Chapter 2

Literature Review

2.1 SCHIZOPHRENIA AND ITS THERAPY

Schizophrenia remains an enigma that has fascinated the foremost minds of psychiatry and neuroscience for more a hundred of years. An understanding of the mechanisms by which the brain filters, prioritizes and processes the relentless current of information available from the richness of its internal, social and natural environments is a key to schizophrenia research.

2.1.1 Definition

The National Institute of Clinical Excellence (NICE) defines Schizophrenia as a brain disorder that produces abnormal thoughts, emotions, perceptions (recognition), behavior, and movement. Schizophrenia is not a "split/multiple personality" nor is it a result of a bad upbringing, weakness, or laziness. It affects approximately 1% of the world population. The onset is usually in the late teens to mid-30s with women having a slightly later onset than men (average age late 20s versus early to mid-20s). There is no gender or racial differences. A graphical representation of the etiology is shown in figure 2.1

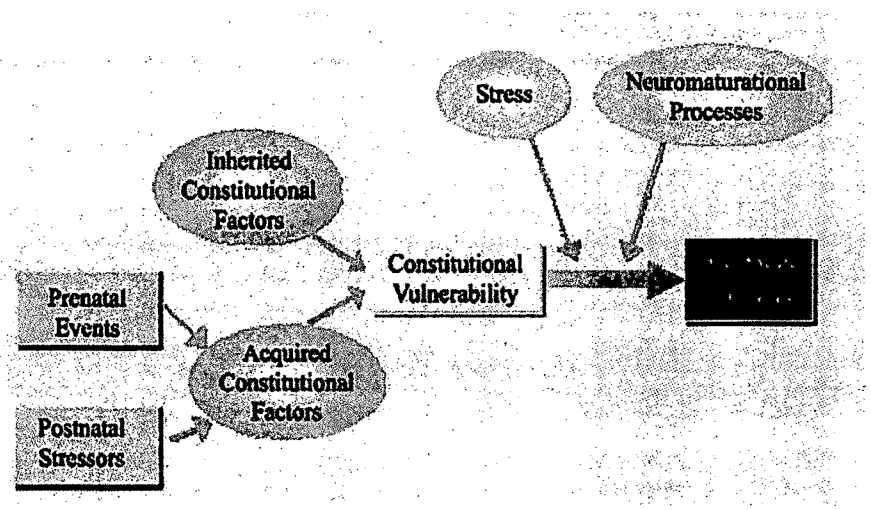


Figure 2.1: Etiology of Schizophrenia

2.1.2 Symptoms of Schizophrenia

Positive Symptoms: Delusions, hallucinations, disorganized speech, unusual behavior, agitation.

Negative Symptoms: Lack of emotion, inability to speak, lack of motivation, no pleasure from activities that would normally be fun, slow movements.

Symptoms Involving Thoughts: Decreased attention span and memory, difficulty making decisions, trouble using well-known skills.

Mood: Depression, unpleasant feelings, hopelessness, low self-esteem.

Social and Job Difficulties: Social isolation, difficulty holding a job, lack of attention to appearance, difficulty maintaining relationships.

Subtype	Characteristics
Paranoid	A preoccupation with one or more delusions or frequent auditory hallucinations
Disorganized	Disorganized speech and behavior and a flat or inappropriate affect are all prominent
Catatonic	A lack of motor response to a stimulus, excessive motor activity, absence of speech, peculiar movements and repetitions of words and phrases or others movements
Undifferentiated	Symptoms of schizophrenia are present but conditions for all other three types are not meet
Residual	Absence of prominent delusions, hallucinations, disorganized speech and grossly disorganized and catatonic behavior despite continuing evidence of a disturbance

Table 2.1: The five principle subtypes in the spectrum of psychiatric disorders

Most individuals with schizophrenia are not violent; more typically, they are withdrawn and prefer to be left alone. Substance abuse significantly raises the rate of violence in people with schizophrenia but also in people who do not have any mental illness. The subtypes of schizophrenia are shown in table 1. People with paranoid and psychotic symptoms, which can become worse if medications are discontinued, may also be at higher risk for violent behavior. When violence does occur, it is most frequently targeted at family members and friends, and more often takes place at home. Suicide is a serious danger in people who have schizophrenia. People with schizophrenia have a higher rate of suicide than the general population. Approximately 10 percent of people with schizophrenia (especially younger adult males) commit suicide. Unfortunately, the prediction of suicide in people with schizophrenia can be especially difficult.

2.1.3 Diagnosis of Schizophrenia

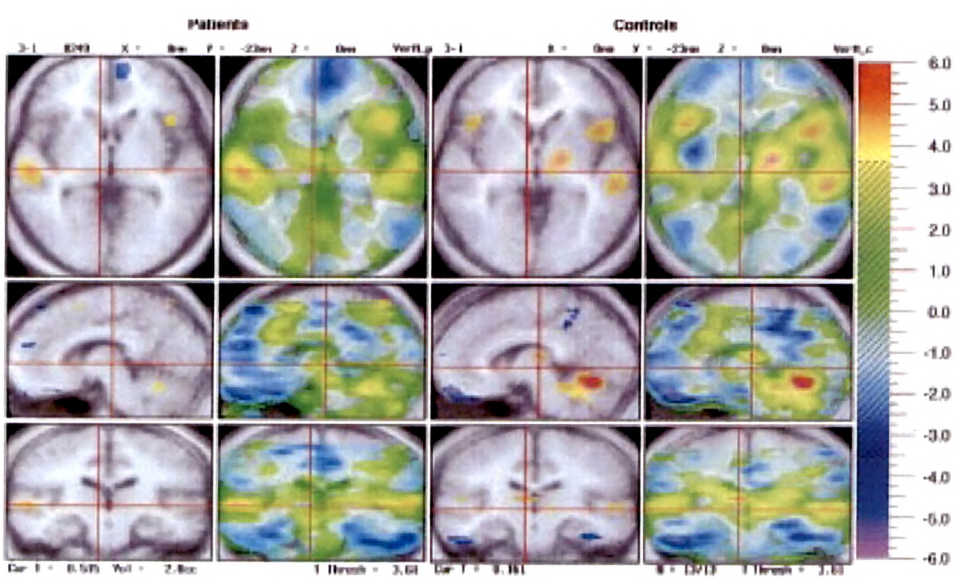


Figure 2.2: PET scans of some schizophrenic patients and their controls

A very common misconception in families of people with schizophrenia is the though that "if we do nothing, maybe it will get better - maybe it's just a phase." The truth is, however, that this is typically the worse thing that can be done if the person does have schizophrenia, and greatly increases the probability that the person will suffer much more permanent damage than

if treated quickly. Schizophrenia is generally recognized now as a disease of the brain (with significant data that supports the belief that it is a neurodevelopment problem in the brain) in which the brain is physically damaged and unfortunately the noticeable symptoms of schizophrenia are usually quite late in the disease process. The risks of developing schizophrenia are shown in figure 2.3

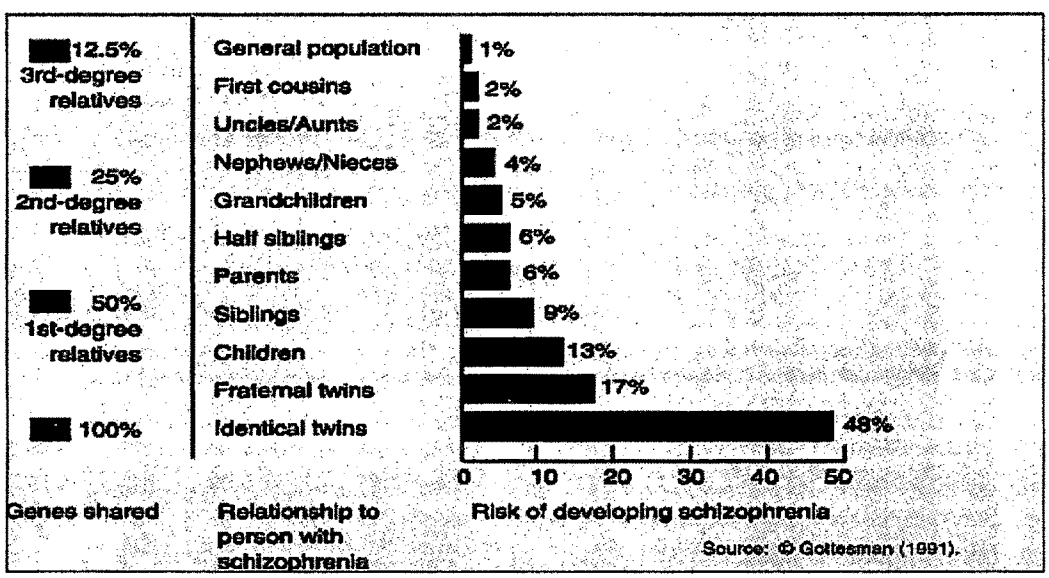


Figure 2.3: Risks of developing Schizophrenia

Individuals who are at risk for developing a psychotic illness usually experience mental and emotional changes before more serious symptoms develop. These early signs are often non-specific, sometimes, even barely noticeable. The unexpected decline in a person's usual way of functioning or relating to others is the most common indicator of an early sign of risk. This early period is called the "prodromal" period (or Prodrome) by psychiatrists. The various stages in psychosis development is shown in figure 2.4

If any of the early signs of risk are present, it is important to seek help quickly in order to ensure the greatest chance for recovery. By identifying and treating the early signs of risk, it is hoped that a psychotic episode might be delayed, prevented, or reduced in intensity. Added to the predisposition towards delays in getting treatment is the fact that as many as 50% of people with schizophrenia can't understand that they are ill (because the part of the brain affected by schizophrenia is frequently the same part that is responsible for self-analysis) and you have a

situation where most people with schizophrenia have a much worse outcome than what is possible given today's treatment options.

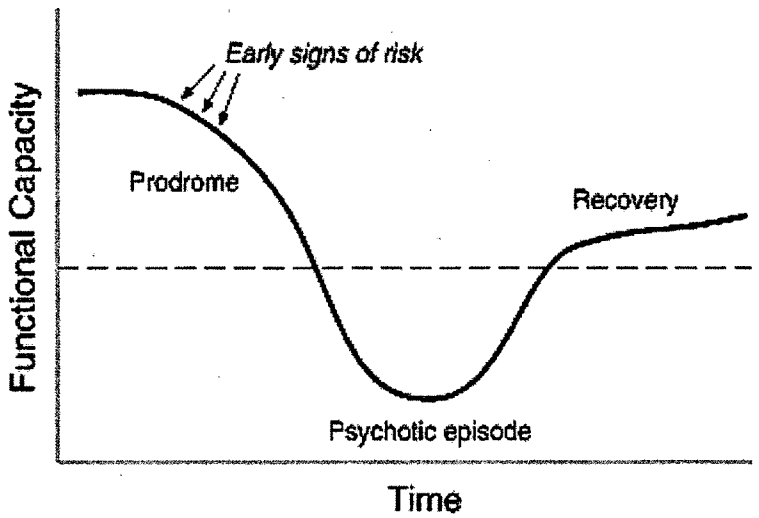


Figure 2.4: Stages of psychosis development

Investigators using magnetic resonance imaging (MRI) have found an association between the occurrence of auditory hallucinations and decreased size of the superior temporal gyrus. Several very recent studies have used positron emission tomography (PET) technology and ingenious experimental designs to extend these early findings. One Such research hypothesizes that people who suffer from schizophrenia have difficulties in "self-monitoring," that are, distinguishing the boundary between their own experiences and what is happening outside their minds. This group argues that a defect in self-monitoring could lead a false attribution of internal thoughts to the outside world, which could explain why people with schizophrenia have hallucinations. To test this idea, they have done a series of studies in healthy volunteers and in people with schizophrenia with and without hallucinations. They gave a group of healthy volunteers an opening of a sentence, such as "*Philip walked down the street and saw...*," and asked them to imagine the sentence being completed in another person's voice. Images from the PET scanner showed this task to activate regions in the temporal and inferior frontal lobes and supplementary motor area, which are responsible for speech monitoring and production. When they compared people with schizophrenia who had hallucinations to

schizophrenic patients who did not hallucinate, they found the hallucinators to have decreased blood flow in the speech monitoring regions as they performed this task. In another study, however, they examined patients during the actual occurrence of hallucinations. They observed additional activations in subcortical (e.g. thalamus) and limbic (e.g. anterior cingulate) regions, as well as the cerebellum. They speculate that these regions could be the "generator" of the hallucinations, while the particular type of hallucination might vary depending on the cortical region engaged (e.g., temporal cortex for auditory hallucinations, visual cortex for visual hallucinations.) This set of findings suggests that localizing the symptoms of schizophrenia may not be quite as simple as localizing right-sided paralysis and impaired speech in a person who has suffered a stroke. Instead, these findings, particularly when coupled with the work of other investigators, suggests that complex approaches to localization may be necessary to explain the diversity of symptoms in schizophrenia.

2.1.4 Theories explaining causes of schizophrenia

2.1.4.1 The Dopamine Theory

The involvement of dopamine in schizophrenia was suggested by the observation that medications that alleviate the psychosis of schizophrenia such as chlorpromazine act by antagonizing the actions of dopamine at its receptor. One such report proposed that dopamine systems mediate the comparison of observed and expected patterns of events within resonant cortical circuits, measuring departures from normality as deviations from patterns of expected resonance. By antagonizing the dopamine receptors, it is possible to block these perceived shifts and the attendant projections of "nonexistent" perceived events (hallucinations) during the active phase of schizophrenia.

2.1.4.2 The Glutamate Theory

Much of the transmission of excitatory information in the brain occurs via the binding of glutamate to its receptors, and, or indirectly, the activity of these excitatory neurons are affected by this amino acid. The blockade of one particular glutamate receptor, the NMDA receptor plays a vital role in the plasticity of the nervous connections associated with learning and memory and appears to mimic certain symptoms of schizophrenia. Moreover, two popular psychoactive drugs (phencyclidine and ketamine) specifically block NMDA receptor and were known to produce positive and negative symptoms in humans. In light of these observations,

one theory was put forward stating that schizophrenia results from a hypoactivity of glutaminergic transmission in the brain. A decrease in the glutaminergic output from the hippocampus, couples with the high levels of glutamate receptors in the anterior cingulate cortex, may underline some of the diminished cognitive aspects and processing deficits associated with schizophrenia.

2.1.4.3 The Acetylcholine Theory

It is was notable that the prevalence of smoking amongst schizophrenics is 3 times higher than the general people in the United States (20-25%), as over 75% schizophrenics smoke. Further more nicotine withdrawal may temporarily worsen schizophrenic symptoms, suggesting that nicotine may help to control psychotic symptoms. The specificity of nicotine in the actions upon a subtype of "fast" acetylcholine receptors, known for their medication of rapid excitatory signals, especially within the presynaptic terminal, infers a central role for the acetylcholine in the etiology of schizophrenia.

2.1.4.4 The Serotonin Theory

The actions of clozapine, an atypical antipsychotic used in the treatment of indiduals resistant to dopamine antagonists is noted to have a high affinity for serotonin receptors, suggested that serotonin may play a role in the etiology of schizophrenia. Fluoxetine, an antidepressant, as well as other selective serotonin reuptake inhibitors (SSRIs) are alleged to cause long term deficits in memory, concentration and even mental disability, disrupting perceptions of reality and creating false memories. Elevated serotonin levels have been found in schizophrenia and SSRIs are alleged to have created an epidemic of suicide attempts.

2.1.4.5 The GABA Theory

Benzodiazepines act by increasing the efficacy of transmission through GABA receptors and which are used both as sedative and in the treatment of anxiety is also shown to ameliorate the core positive symptoms of schizophrenia. The brains of cocaine addicts, who exhibit schizophrenia symptoms, are more sensitive to benzodiazepines than those of drug free individuals, indicating a change in GABA pathway in these individuals. GABA receptors have further been shown to be important in the acquisition of behavioral sensitization to drugs that induce schizophrenia like behaviors, such as metamphetamine.

2.1.5 Current management of schizophrenia

Antipsychotic drug therapy remains the main method for treatment of schizophrenia. Antipsychotic agents are classified as typical antipsychotics and atypical antipsychotics. All typical antipsychotics are dopamine antagonists (Kane JM., 1996) and were the main stay of drug treatment for Schizophrenia and other psychotic disorders. The non-selective dopamine [D_2] receptor blockade of typical antipsychotics is responsible for the commonly observed side effects. Atypical antipsychotics on the other hand, block both central serotonin [$5HT_2$] and dopamine [D_2] receptors. Concurrent blockade of both these receptors is thought to be effective in diminishing the severity of extra pyramidal side effects and in improving negative symptoms. Binding of these antipsychotic agents to the dopamine receptors in the nigrostriatal pathway are responsible for Parkinsonism and other extra pyramidal (EPS) side effects. Unfortunately, muscarnic receptor antagonism is responsible for the well known adverse side effects like blurred vision, dry mouth, sinus tachycardia, constipation, urinary retention etc.

Antipsychotics may also block α_1, α_2 adrenoreceptors and histamine- 1 receptors, which contribute to the side effect profile. Blocking α_1 receptors can cause postural hypotension, dizziness and reflux tachycardia. Blocking of α_2 receptor can antagonize the hypertensive effects of clonidine, methyldopa or guanabenz. Histamine-1 receptor blockade can cause sedation, which may or may not be a desired effect and weight gain. The relative receptor affinities of some commonly used antipsychotic agents are shown in table 2.2

Clinical trials have clearly indicated the superiority of atypical antipsychotics on comparison with the typical antipsychotics in medication adherence behavior and quality of life (Nicola O'Connell, 2000), showing 28% improvement in the medication adherence with patients on atypical antipsychotic therapy (Vorugini LNP et al., 2002). Another similar study with larger sample of out patients followed up 3 months after discharge reported a trend towards improved medication adherence behavior for patients with atypical antipsychotics compared with those on typical antipsychotics (Olfson M., 2000).

Receptor	Ziprasidone	Risperidone	Olanzapine	Clozapine	Haloperidol
D_2	High	High	Moderate	Low	High
$5HT_{2A}$	High	High	Moderate	Moderate	Low
α_1 adrenergic	Moderate	Moderate	Low	Moderate	Low
α_2 adrenergic	Low	High	Low	High	Low
Muscarnic 1	Negligible	Negligible	High	High	Negligible
Histaminic 1	Moderate	Moderate	High	High	Low

Table 2.2: Relative receptor affinities of some antipsychotics

Antipsychotic medications reduce the risk of future psychotic episodes in patients who have recovered from an acute episode. Even with continued drug treatment, some people who have recovered suffer relapses. Far higher relapse rates are seen when medication is discontinued. In most cases, it would not be accurate to say that continued drug treatment "prevents" relapses; rather, it reduces their intensity and frequency. The treatment of severe psychotic symptoms generally requires higher dosages than those used for maintenance treatment. If symptoms reappear on a lower dosage, a temporary increase in dosage may prevent a full-blown relapse. Because relapse of illness is more likely when antipsychotic medications are discontinued or taken irregularly, it is very important that people with schizophrenia work with their doctors and family members to adhere to their treatment plan. Adherence to treatment refers to the degree to which patients follow the treatment plans recommended by their doctors. Good adherence involves taking prescribed medication at the correct dose and proper times each day, attending clinic appointments, and/or carefully following other treatment procedures. Treatment adherence is often difficult for people with schizophrenia, but it can be made easier with the help of several strategies and can lead to improved quality of life. The brand names, doses and dosage forms available for some selected antipsychotic agents are tabulated in table 2.3

Nonproprietary name	Trade name	Oral Dose (mg)	Dosage form	Side effects		
				Sedative	EPS	Hypotensive
Chlorpromazine hydrochloride	THORAZINE	200 - 800	O, SR, L, I, S	+++	++	IM +++ Oral ++
Mesoridazine besylate	SERENTIL	75 -300	O,L,I	+++	+	++
Thioridazine Hydrochloride	MELLARIL	150 – 600	O,L	+++	+	+++
Fluphenzaine Hydrochloride	PREMITIL	2 – 20	O, L, I	+	++++	+
Perphenazine	TRILAFON	8 – 32	O,L,I	++	++	+
Trifluoperazine hydrochloride	STELAZINE	5 – 20	O, L, I	+	+++	+
Thiothixene hydrochloride	NAVANE	5 – 30	O, L, I	++	+++	++
Clozapine	CLOZARIL	150 – 450	O	+++	0	+++
Haloperidol	HALDOL	2 – 20	O,L,I	+	++++	+
Loxapine succinate	LOXITANE	60 – 100	O,L,I	+	++	+
Olanzapine	ZYPREXA	5 – 10	O, I	+	+	++
Pimozide	ORAP	2 -6	O	+	+++	+
Quetiapine Fumarate	SEROQUEL	300 – 500	O	+++	0	+++
Risperidone	RISPERDAL	2 -8	O, I	++	++	+++
Ziprasidone	GEODON	80- 160	O, I	++	+++	+

Side Effects: *0: Absent; +: low; ++: moderate; +++: moderately high; ++++: high*

Dosage forms: *I – regular or long acting injections; L – Oral liquid or oral liquid concentrate; O – Oral solid; S – Suppository; SR – Oral sustained release*

Table 2.3: Selected antipsychotic drugs: Brand Name, doses and dosage forms available

2.2 DRUG DELIVERY TO THE BRAIN

2.2.1 The blood brain barrier and its properties

The blood–brain barrier (BBB) is created by the endothelial cells forming the capillaries of the brain. The endothelial cells are linked at their lateral surfaces by tight junctions formed by a number of transmembrane proteins, which constitute an effective block to aqueous diffusion between the cells (Brightman MW and Reese TS, 1969; Kniesel U and Wolburg H, 2000). Thus, unlike capillaries in the peripheral circulation, there are no para cellular pathways available for the diffusion of aqueous solutes from the blood to the brain extra cellular fluid or in the reverse direction.

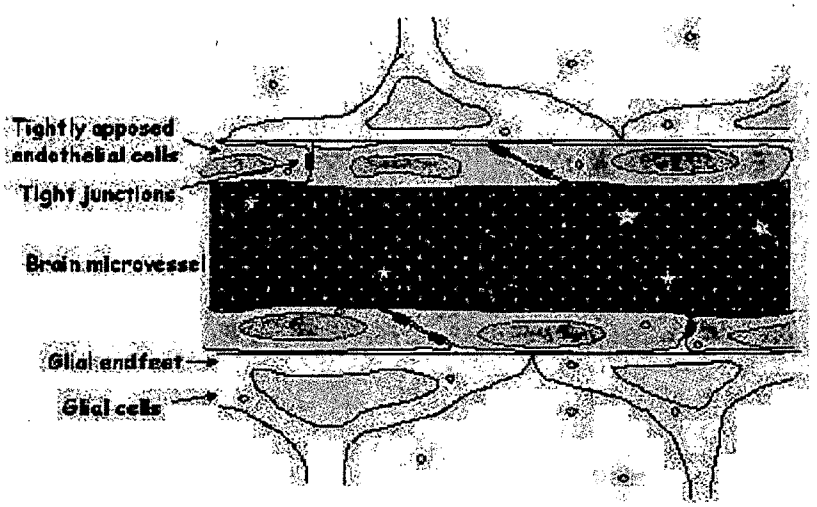


Figure 2.5: Diagrammatic representation of the blood brain barrier

The creation of the BBB in this way effectively seals off the brain to access by polar blood-borne solutes. Many of these polar solutes are essential nutrients and metabolites for the brain, such as glucose and amino acids, and therefore the endothelial cells of the cerebral capillaries must maintain a high level of expression of transporters for these essential solutes in order to facilitate their entry into the brain. Thus, the tight junctions form an effective gate in the potential para cellular diffusional pathway. The tight junctions probably also act as a fence in the cell membrane, in that they prevent the free movement of transmembrane proteins and lipid rafts in the cell membrane between the luminal and the abluminal surfaces of the endothelial cells. This fence property of the tight junctions enables the maintenance of a polar expression

of many transporters present in the membranes of the cerebral endothelial cells and allows some transporters to be expressed solely in the luminal membrane and some in the abluminal membrane. Thus, as a result of this polarized transporter expression, for a range of solutes, transport can be primarily directed from blood to brain and for other solutes in the opposite direction, thus removing them from the central nervous system (CNS). This differentiation of the cerebral endothelial cells to form a tight polarized BBB, rather than an open endothelium seen in other tissues, is thought to be the result of a close cellular association with both astrocytes and pericytes, both of which are closely applied to the basement membrane surrounding the abluminal surface of the cerebral capillaries (Kacem K et al., 1998; Dore Duffy P., 2003).

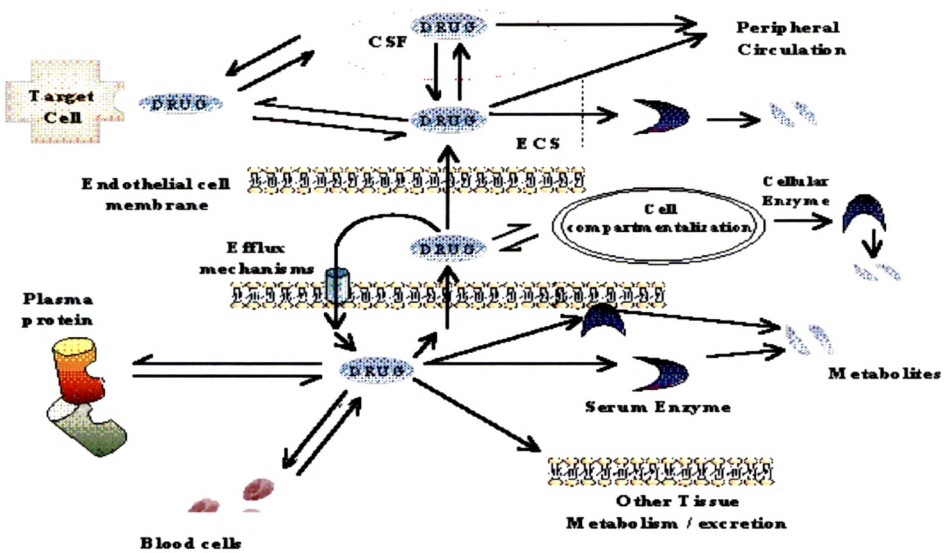


Figure 2.6: Potential factors which alter drug uptake into the brain

The astrocytes, the cell bodies of which are situated in the brain parenchyma, have end feet, which project down to the cerebral capillaries and spread in a network over the abluminal surface. Although there is still considerable debate about the factor, or factors, which may induce the very specific differentiation of the BBB endothelium, there are probably both cell surface molecules (cell–cell contact) and soluble factors involved. The more lipophilic

substances that are present in blood can diffuse passively directly through the lipid of the cell membrane and enter the endothelial cells and brain by this means.

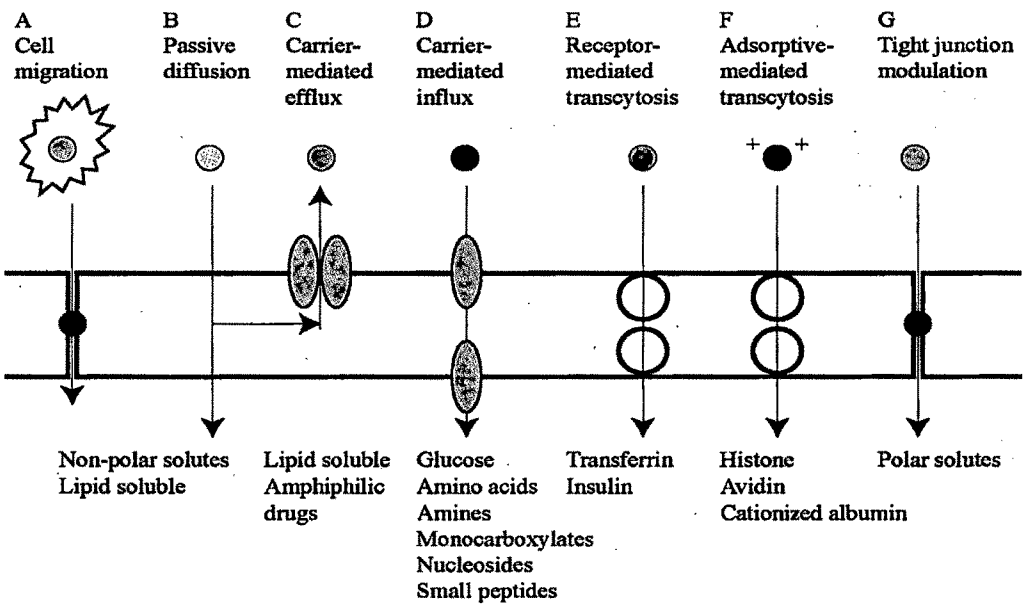


Figure 2.7: Transport routes across the blood–brain barrier (BBB) (A) Leukocyte movement across the BBB (B) Molecules may diffuse across the BBB by passive diffusion through the cell membranes and across the endothelial cells. In general, the more lipid soluble a molecule the greater the CNS penetration (C) Active carrier-mediated efflux transporters are able to expel a wide and varied range of molecules out of the BBB and reduce their CNS penetration to a level below that predicted by their lipid solubility (D) Many necessary polar metabolites and nutrients must be carried into the CNS by transporters in the BBB (E) Some macromolecules, proteins and peptides are transported by receptor-mediated transcytosis (F) Other macromolecules, especially cationic macromolecules, induce adsorptive-mediated transcytosis (G) Tight junctions may be modulated to allow an increased movement of polar solutes through the aqueous para cellular pathway (Begley D and Brightman MW, 2003)

In general, there is a relationship between lipophilicity and brain penetration, and the more lipid soluble a molecule, the more readily it will enter the CNS (Levin VA., 1980). Some of these lipophilic substances do not, however, enter the brain as readily as one might expect or predict from their lipid solubility. These solutes, and in many cases their metabolites, are actively removed from the CNS by efflux transporters (Begley D, 2003). The capacity of the cerebral endothelial cells, the pericytes, the choroid plexuses and brain tissue to metabolically transform drugs has probably been underestimated (Minn A et al., 2000). Most electron microscopical studies of the BBB indicate a limited endocytotic activity compared with peripheral endothelial cells (Begley D and Brightman MW, 2003). An electron micrograph is an instant snapshot of a dynamic event and is therefore difficult to quantify. However, receptor mediated and absorptive-mediated transcytosis occur in the BBB and, under suitable conditions, transcytosis forms a significant route across the BBB for macromolecules and peptides.

The BBB has two major functions.

Firstly, the internal environment of the brain, the brain interstitial fluid (ISF) and the cerebrospinal fluid (CSF) composition have to be controlled within extremely fine limits, far more so than the somatic extra cellular fluid, so that neurones can perform their complex integrative functions. This integrative neural function of the CNS relies almost solely on accurate synaptic transmission and spatial and temporal summation. Some amino acids in the blood, that are present in high concentrations, such as glycine, glutamic acid and aspartic acid, are potent excitatory neurotransmitters in the CNS and their levels in brain extra cellular fluid must be very precisely controlled. Central neuronal synapses require this very stable background against which to function (Begley D and Brightman MW, 2003).

Secondly, a major function of the BBB is that of neuro protection. Over a lifetime the CNS will be exposed to a wide range of neurotoxic metabolites and acquired xenobiotics, which may cause cell damage and death. As neuronal replacement is virtually absent in the CNS of mammals, a phylogenetic class of animal where individuals may have a relatively long lifespan, any enhancement of neuronal death will accelerate degenerative pathologies and advance natural debilitation with age. The efflux transporters of the BBB previously mentioned are particularly important in this respect (Begley D and Brightman MW, 2003). A direct consequence of the existence of the BBB is the clinical difficulty in delivering therapeutic

compounds to the CNS. Polar drugs cannot diffuse in freely, as the para cellular pathway is absent, and a very large number of the more lipophilic drugs are subject to the activity of efflux transporters. Thus, a significant number of CNS diseases have poorly met therapy largely due to the difficulty of delivering drugs across the BBB.

CNS diseases
Alzheimer's disease
Amyotrophic lateral sclerosis
Brain cancer
Canavan's disease
CNS trauma
Creutzfeldt–Jacob disease
Fragile X syndrome
Huntingdon's chorea
Inherited ataxias
Inborn metabolic errors
Lysosomal storage diseases
Multiple sclerosis
Neurological AIDS
Parkinson's disease
Stroke

Table 2.4: CNS diseases in which therapy is poorly effective, largely due to the difficulty of delivering drugs across the blood–brain barrier

The BBB is the single most significant factor limiting drug delivery to the CNS. The capillary network within the brain is very dense, having a surface area between 100 and 200 cm^2/g of brain depending on the brain region. Consequently, most brain cells, neurones or astrocytes, are directly no more than 20μm from a capillary (Pardridge WM., 1991). Because of the cellular complexity of the neuropil, the extra cellular pathways in brain parenchyma are tortuous, and the true aqueous diffusional distances for more polar solutes may be greater than 20μm. However, once through the BBB, a drug or solute is very close to its potential site of

action. A major factor that limits the half-life in the cerebral compartment of a solute or a brain-targeted drug is the secretion of brain ISF and CSF (Begley D, 2003). The ISF is secreted by the cerebral endothelium and the major part of the CSF by the choroid plexuses. In the brain, ISF is secreted at the rate of 0.17µl/g of brain (Cserr HF and Patlak CS., 1992; Cserr HF et al, 1981). This rate of secretion is probably similar for all mammalian species, as the capillary density is comparable for the same brain region between different species.

Also, the secretion of fluid is driven by the sodium/potassium ATPase, located on the abluminal membrane of the endothelial cells (Betz AL et al., 1980), which probably has a very similar interspecies expression/activity in the BBB. As the extra cellular space of the brain is approximately 200µl/g of tissue (20%), this means that the interstitial volume will be replaced every 20 hours (Begley D et al., 2000). Thus, diffusional times/distances may not be the major factor in the extra cellular space of the neuropil, as movement in the intercellular spaces will be largely driven by ISF secretion and bulk flow. With the CSF the situation is rather different. The choroid plexuses secrete CSF at a rate of about 360–880µl/g of plexus tissue, which means that the CSF is replaced approximately every 4.4 hours in the human (Begley D et al., 2000). In smaller mammalian species, and also human infants, the relative size of the choroid plexus is larger, in relation to the ventricular volume, resulting in the CSF being replaced faster. In the rat, for instance, the CSF is replaced every 2.3 hours (Begley D et al., 2000). Thus, determination of the CSF concentration of a systemically administered drug is likely to be a poor guide to the ISF concentration of the drug and will reflect an even lower relative concentration to the ISF level in a smaller brain. Once drugs are introduced into the cerebral compartment, therefore, their half life is limited by fluid turnover, efflux transporters and metabolism. A number of strategies exist to enhance drug transport across the BBB and are reviewed below.

Lipidization and other physico-chemical manipulations : Most drugs used to treat CNS disease have a molecular weight between 150 and 500 Da and a calculated log octanol/water partition coefficient between -0.5 and 6.0 (Bodor N and Buchwald P., 2003). CNS penetrance is also optimized if they are uncharged at physiological pH, as it is generally assumed that the charged fraction cannot diffuse through the cell membrane. It is, therefore, the diffusion gradient created by the uncharged fraction that drives passive movement through the barrier. The molecular characteristics that reduce a molecule's penetrance through the BBB are a high

polarity with a polar surface area calculated at above 8 nm^2, a high Lewis bond strength and a high capacity for hydrogen bonding. The relationship between lipid solubility and BBB permeability has long been recognized, and BBB penetration for passively penetrating molecules increases in a linear manner with lipophilicity. Certain compounds have a high BBB penetrance that is not commensurate with their lipid solubility. These include metabolites such as glucose and many amino acids that have transporters facilitating the entry of these essentially polar molecules, and a wide diversity of drugs, the lipid solubility of which would predict a larger BBB entry than is observed. Initially, it was thought that drugs in this latter group were all high molecular weight bulky structures and their relative lack of penetrance was attributed to these physical characteristics hindering their diffusion through the cell membrane. It has subsequently been recognized that these and many other compounds are substrates for the efflux transporters P-glycoprotein (Pgp), breast cancer related protein (BCRP) and multidrug resistance proteins (MRPs), all of which are active in the BBB (Begley D et al., 2000).

Pro-drugs and chemical delivery systems: The relationship between lipid solubility and BBB permeability has long been recognized, and BBB penetration for passively penetrating molecules increases in a linear manner with lipophilicity. For example morphine has a very low brain uptake; replacing one hydroxyl group of morphine with methyl group to form codeine increases the lipophilicity. On further lipidization, adding two acetyl groups to the molecule to form heroin (or di-acetyl-morphine), increases the brain uptake to very significant levels. Heroin is rapidly metabolized to morphine in the brain, which interacts with the opiod receptor (Bodor N and Buchwald P., 2003). Morphine being relatively polar becomes effectively locked into the brain as it cannot diffuse back out across the BBB. This lock-in principle is a major feature of the pro-drug approach to CNS delivery.

BBB modulation: Artificially opening the BBB by administration of hyper osmotic agents or vasoactive molecules, e.g., bradykinin, histamine, serotonin is well established (Neuwelt EA et al., 1991; Rapoport SI., 2000). When a hypertonic solution of 25% mannitol is introduced via a cannula into the carotid artery (in humans at a rate of 4–8 ml/second), for approximately 30 seconds the BBB remains open for 30 minutes, Following this treatment, a drug can be administered via the same catheter and will freely enter the CNS. Although compromising

BBB integrity allows for paracellular transport of polar drug molecules into the brain, there is potential danger of toxicity caused by CNS entry of other unwanted molecules.

Intracerebral injection/infusion: An obvious way of circumventing the BBB is to inject a drug directly into the brain, intraventricularly or intrathecally into the subarachnoid space. Diffusion of drug molecules, such as proteins, is limited in the brain tissue. Injection can be of either a solution of the drug or a slow-release implant containing the drug. These methods have a number of drawbacks. If an injected volume or a solid implant is rapidly introduced into brain tissue it will certainly damage an area of brain (Krewson CE et al., 1995; Yan Q et al., 1994).

The olfactory route: The rapid CNS transport of many solutes via the nasal route probably underlies the relative popularity of cocaine sniffing. It is probably significant that the olfactory neurons are one of the relatively few groups of central neurons that are capable of regeneration, presumably as a result of their high attrition rate caused by continual exposure to environmental agents. Intranasal administration has been proven to allow for uptake of some molecules into the brain. The olfactory nerve pathway allows bypassing the BBB; Certainly, large peptides such as insulin, and even viruses, can achieve entry to the CNS via the nasal route, and this pathway may form an important channel of access for meningeal and other CNS infections (Illum L., 2003; Mathison S et al., 1998).

Delivery via endogenous transporters: There are a large number of solute transporters or carriers present in the BBB. Many of these carrier-mediated mechanisms are designed to carry polar metabolites into the CNS; Drugs can therefore be designed as pseudo-substrates for these transporters, and thus be carried into the brain. For example the endogenous transporter, α methyl-dopa, has been successfully employed for the transport of large neutral amino acids across the BBB (Tsuji A., 2000; Mertsch K and Maas J., 2000). The drugs which use endogenous transporters to cross the BBB are tabulated in table no 2.5

Endogenous transporter	Category of drug carried
Medium chain fatty acid carrier	Valproic acid
	Docosahexanoic acid (DHA)-taxol
	DHA-ddC
Large neutral amino acid carrier	L-dopa
	α methyl-dopa
	Melphalan
	Baclophen
	Gabapentin
	Activicin
	D,L-NAM
	Phosphonoformate–tyrosine conjugate
	Nitrosoarginine derivatives
Monocarboxylic acid carrier	Active metabolites of simvastin and lovastatin (with carboxylic acid groups)
Organic cation transporter	Mepyramine
	Diphenhydramine
	Diphenylpyraline
	Lidocane
	Imipramine
	Propranolol
	Purine carrier Oxazolamine COR3224
	Nucleoside carrier Abacivir
	Hexose carrier Dehydroascorbic acid
	Glycosylated morphine

DHA-ddC, Docosahexanoic acid-2,3-dideoxycytidine; D,L-NAM, D,L-2-amino-7-bis[(2-chloroethyl)amino]-1,2,3,4-tetrahydro-2-napthoic acid.

Table 2.5 Drugs that can use endogenous transporters to cross the blood–brain barrier

Cell-penetrating peptide vectors: Cell-penetrating peptide vectors offer a further interesting opportunity for enhancing the brain uptake of poorly penetrating drugs. Cell-penetrating peptides are based on either naturally occurring or synthetic peptides that are able to pass directly through a cell membrane and do not require endocytic uptake. These delivery peptides are able to penetrate the cell membrane without a cytolytic effect. For example, two of these peptides – Syn B1 and penetratin have been shown to increase the brain uptake of the anticancer agent doxorubicin six- to eightfold when the drug is attached to the protein (Begley DJ., 1996; Rousselle C et al., 2000). Some cell penetrating peptides and their general properties are tabulated in table no. 2.6

Cell-penetrating peptides	General properties of these peptides
Penetratin RQIKIWFQNRRMKWKK	Amino acids can be D-isomers to limit enzymic degradation
SynB1 RGGRLSYSRRRFSTSTGR	Amphipathic with separated positively charged and hydrophobic domains
HIV1-nuclear transcription activator (fragment 48–60) GRKKRRQRRRPPQ	Repeated amino acid sequences. Engineered to be linear α - helix (no disulphide bond)
pVEC (vascular endothelial cadherin) LLIILRRRIRKQAHAHSK	Penetrate cell membranes without cytolytic effect. Hydrophobic domains may 'worm' into the cell membrane
Transportan GWTLSNAGYLLKINLKALAALAKKIL	Positively charged regions may interact with negatively charged phospholipid heads. Mechanisms of cell penetration may be similar to those of signal peptides. Properties of all the cell-penetrating peptides listed.

Table 2.6: Examples of cell-penetrating peptides and their properties

Inhibition of efflux mechanisms (ABC transporters): The BBB contains a number of active efflux mechanisms, which will, in an ATP- dependent manner, remove many drugs from the CNS. In general these drugs are compounds the physico-chemical properties of which, for example an optimal lipophilicity, small polar surface area and the presence of aromatic rings, also tend to make them good candidates for substrates of efflux transporters. In the BBB, Pgp, BCRP and MRPs 1 and 2 would appear to be the major efflux transporters (Begley D., 2003). A strategy for enhancing the CNS penetration for a number of drugs subject to efflux transporter activity is to develop competitive or non-competitive inhibitors. Table 2.7 shows a number of inhibitors that have been developed for enhancing the uptake of efflux inhibitor substrates, and Table 2.8 lists a number of drugs for which an increase in CNS uptake has been demonstrated using this strategy.

ABC transporter modulators	Drug
PSC833	Vinblastine
	Colchicine
	Digoxin
	Paclitaxel
	Morphine-6-glucuronide
GF120918	Colchicine
	Vinblastine
	2_,3_-Didioxyinosine
	Amprenivir
	Itraconazole
	Paclitaxel
LY335979	Nelfinavir

Table 2.7: Examples of drugs for which increased CNS uptake has been demonstrated using ABC transporter modulators

Generation	ABC transporter modulators	Efflux transporter target
First	Probenecid	MRP1/MRP2
	Sulfinpyrazone	MRP1/MRP2
	Benzbromarone	MRP1/MRP2
	Verapamil	Pgp
	Clyclosporin A	Pgp
	Quinidine	Pgp
Second	SDZ-PSC833	Pgp
Third	GF120918	Pgp/BCRP
	LY335979	Pgp
	V-104	Pgp
	Pluronic L-61	Pgp
	Fumitremorgin C	BCRP
Experimental agents	Monoclonal antibodies	
	Liposomes/nanoparticles	
	Oligonucleotides	

Pgp - P-glycoprotein; BCRP - breast cancer related protein; MRPs - multidrug resistance proteins.

Table 2.8: ABC transporter modulators improve the CNS penetration of some compounds by inhibiting the active efflux transporters that usually remove them from the CNS.

Liposomes and nanoparticles: Two innovative strategies for delivering drugs across the BBB are the use of liposomes and nanoparticles. These are complex constructs into which large quantities of drug, peptide or enzyme can be packaged. The surface of the construct can be engineered so that the complex can be targeted to the CNS via specific mechanisms. Pegylated immuno-liposomes have been successfully employed to target and non-permanently transfect

β-galactosidase and luciferase to brain tissue (Pardridge WM, 2002). The gene is encapsulated within the liposome and the liposome is then coated with polyethylene glycol (PEG) to reduce uptake by the reticulo-endothelial system. About 2% of the PEG strands then have a monoclonal antibody to the transferrin receptor (8D3 Mab) attached to them.

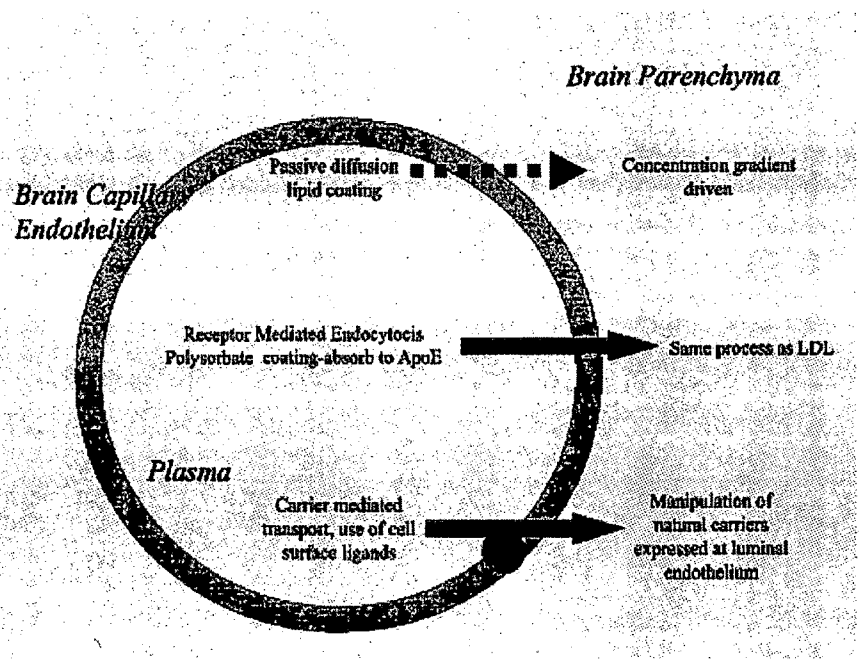


Figure 2.8: Mechanism of nanoparticle entry into the brain

The monoclonal antibody directs the liposome to transferrin receptor-rich tissues, such as brain, liver and spleen. However, if the β -galactosidase gene is coupled with a brain-specific promoter, for example the glial fibrillary acidic protein (GFAP) promoter, then genes for β-galactosidase and luciferase have been successfully targeted to the brain and expressed (Shi N et al., 2001). The transfection is not permanent and peaks at 2 days when the GFAP promoter is used. Presumably, the initial event is endocytic, as with vector constructs consisting of peptides coupled to a transferring receptor Mab. Similar immunoliposome constructs using an OX26 transferrin receptor Mab have also been used to deliver digoxin to the CNS (Huwyler J et al., 2002). In this case, digoxin is also a Pgp substrate and appears to be carried past the Pgp efflux transporter by the immunoliposome and thus effectively bypasses the efflux mechanism. Poly (butyl) cyanoacrylate (PBCA) nanoparticles have also been successfully used to deliver a

wide range of drugs to the CNS (Kreuter J., 2001). PBCA nanoparticles are typically 250 nm in diameter. Drugs may be either incorporated into the particle structure or absorbed onto the surface. The particles are then coated with Tween-80 which, when the drug-loaded particles are injected into blood, appears to cause a further binding of apolipoprotein E to the surface of the particle. This final product is then thought to be mistaken for a low density lipoprotein (LDL) particle by the cerebral endothelial cells and is subject to endocytosis by the LDL transport system (Kreuter J et al., 2002; Kreuter J et al., 2003).

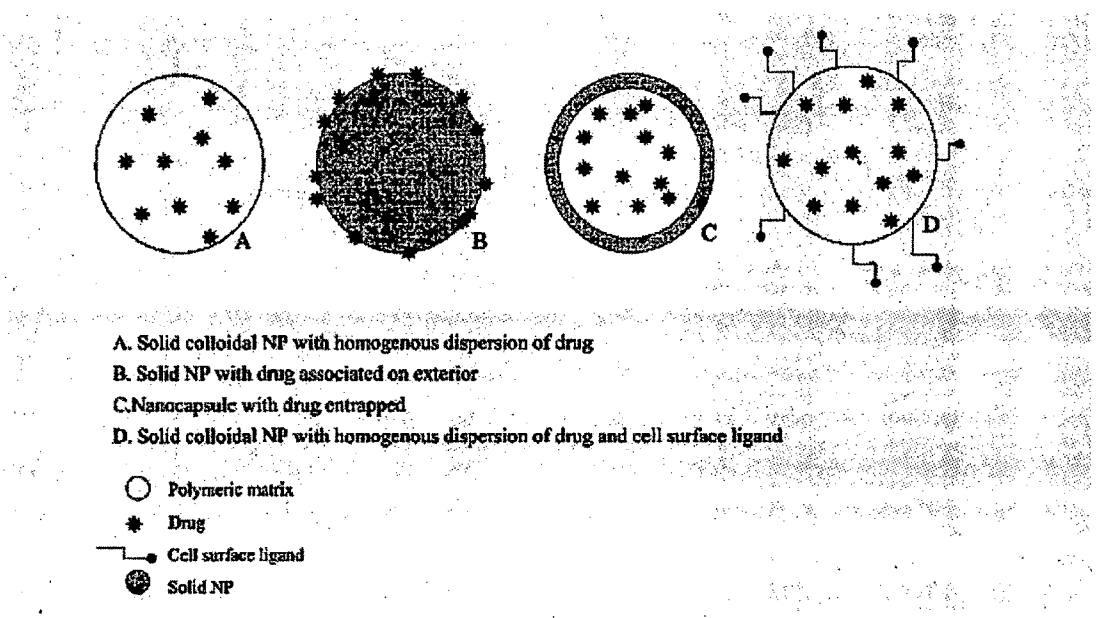


Figure 2.9: Types of nanoparticles for CNS delivery

A number of the drugs delivered to the CNS by PBCA nanoparticles are Pgp substrates; thus, the nanoparticles also seem capable of bypassing Pgp. The PBCA nanoparticles are clearly endocytosed by the cerebral endothelial cells (Ramge P et al., 2000; Alyaudtin RN et al., 2001), but again the events following the initial cell uptake leading to the transfer of drug into the brain are still unclear. The great advantages with both immunoliposome and nanoparticle drug delivery to the CNS are that very complex constructs can be put together for site-specific delivery, high molecular weight molecules can be delivered and large drug payloads are possible.

2.3 P-GLYCOPROTIEN

The drug efflux transporter P-glycoprotein (P-gp) was originally discovered by its ability to confer multidrug resistance (MDR) to tumor cells. Juliano and Ling (Juliano RL and Ling V., 1976) were the first to report the presence of Pgp in 1976. Subsequently, numerous other laboratories (Inaba M and Johnson RK., 1977; Inaba M et al., 1979; Beck WT., 1987) have confirmed that many tumor cell lines over expressed with P-gp exhibit reduced intracellular drug concentration secondary to enhanced drug efflux. When the MDR1 gene that encodes P-gp was transfected in drug sensitive cells, these transfectants became drug-insensitive cells and exhibited low intracellular drug concentration. The role of P-gp in multidrug resistance was further confirmed by Tsuruo and colleagues (Tsuruo T et al., 1981) who demonstrated that verapamil (a potent P-gp inhibitor) increased the sensitivity of multidrug-resistant leukemia cells to anticancer drugs. From these results, it is evident that P-gp plays a role in multidrug resistance to cancer chemotherapy.

2.3.1 Structure of P- Glycoprotein

The isolation of cDNAs encoding the P-glycoprotein enabled analysis of the structure of the polypeptide. The nucleic acid sequences of the cDNAs which encode the mammalian P-glycoproteins predict an integral membrane glycoprotein with remarkably similar structures (Figure 2.8). The length of the mammalian P-glycoproteins varies from 1276 to 1281 amino acids predicting a molecular weight of approximately 140 kD. This is consistent with purified or labeled P-glycoprotein which migrates at a molecular weight of 130–180 kD in polyacrylamide gels. Although P-glycoprotein mobility in polyacrylamide gels is sensitive to the conditions employed (Greenberger LM et al., 1988a), approximately 10–15 kD of the observed molecular weight is accounted for by N-linked glycosylation (Greenberger et al., 1988). Indeed, the predicted amino acid structure of the P-glycoprotein indicates 7–10 potential N-linked glycosylation sites [N-X-S/T]. Examination of the amino acid sequence identifies a number of significant structural features. Independent hydrophobicity analyses using the methods of Kyte-Doolitte (Gros P et al., 1986) and Eisenberg (Chen CJ et al., 1986) predict a polypeptide which traverses the plasma membrane 12 times. A short, highly charged, cytoplasmic domain precedes three membrane loops which are followed by a large cytoplasmic domain. A cluster of potential N-linked glycosylation sites are consistently located in the first external loop in virtually all P-glycoproteins. The large cytoplasmic domain includes the paired consensus sequences G-x(4)-G-K-[ST] and [RK]-x(3)-Gx(3)-L-

[Hydrophobic](3) separated by approximately 120 amino acids which function as a nucleotide binding fold in a wide range of ATPases (Walker JE et al., 1982). The cytoplasmic domain is followed by three additional membrane loops and another large cytoplasmic domain with the consensus sequence for a second nucleotide binding fold. Experimental evidence indicates that the P-glycoprotein binds and hydrolyzes ATP (Ambudkar SV et al., 1992; Sarkadi B et al., 1992) which is required for drug transport (Horio M et al., 1988). This model of the P-glycoprotein suggests that it is comprised of two halves, each with a similar structure. A comparison of the amino acid sequence of the carboxy and amino halves indicates approximately 40% identical and an additional 25% conserved amino acid homology which has suggested duplication of an ancestral gene. The highest similarity between the moieties surrounds the nucleotide binding folds in the large cytoplasmic domains with almost 60% amino acid identity with an additional 20% conserved substitutions. When the two halves of the P-glycoprotein are aligned, a short segment termed the 'linker' region (Van der Bliek AM et al., 1987) which does not readily align with the corresponding region in the proximal half of the polypeptide appears to bridge the amino and carboxy halves. This highly charged region is capable of self annealing in vitro and has been posed to play a role in the potential dimerization of P-glycoprotein (Juvvadi SR et al., 1997). The linker regions of the class I and class II P-glycoproteins, which are capable of conveying drug resistance, each contain consensus sequences for cAMP- and cGMP dependent protein kinase phosphorylation sites, while the class III members do not (Hsu S et al., 1989). Similar sites are found in the first cytoplasmic domain of the two human P-glycoproteins but not in the murine or hamster homologs.

Investigations of the membrane orientation of P-glycoprotein using cell free translation/translocation systems and fusion proteins in bacteria have suggested that multiple topological forms may be present. Polypeptides with four transmembrane domains in either or both the amino half or the carboxy half have been proposed which is distinct from the six transmembrane domains predicted in each half of the protein by the hydropathy analysis. A soluble, heat-labile and trypsin-sensitive activity present in wheat germ extract appeared to modulate different topological structures (Zhang JT and LingV., 1995). These results are contrasted by P-glycoprotein topology mapping based on the insertion of a hemaglutinin epitope in the predicted intra- or extra-cellular domains (Kast C et al., 1996). Immunological localization of the hemaglutinin epitope on functionally expressed P-glycoprotein is consistent only with the twelve transmembrane spanning domain model predicted from the hydropathy

analysis. Insight on the structure of P-glycoprotein has recently been revealed by electron microscopy and single particle images and Fourier projection lamps of 2-dimensional crystalline arrays. The initial interpretation of this data suggests that the P-glycoprotein is a cylinder of approximately 10 nm in diameter with one half of the molecule in the lipid bilayer and the remainder above and below the membrane. There is a large central pore of approximately 5 nm in diameter surrounded by a roughly hexagonal array of the membrane spanning segments. The pore is aqueous and larger than is required for the passage of known substrates. It appears to be closed at the cytoplasmic face by the two intracellular domains which contain nucleotide binding folds. The large size of the pore may explain why the nucleotide binding domains are accessible from the outside of the cell (Baichwal V et al., 1993). Attenuated total reflection infrared spectroscopy analysis similarly indicated that a large fraction of the P-glycoprotein is poorly accessible to the aqueous phase and that Mg ATP, but not ligands, induced tertiary structural changes which increased accessibility to this compartment (Sonveaux N et al., 1996).

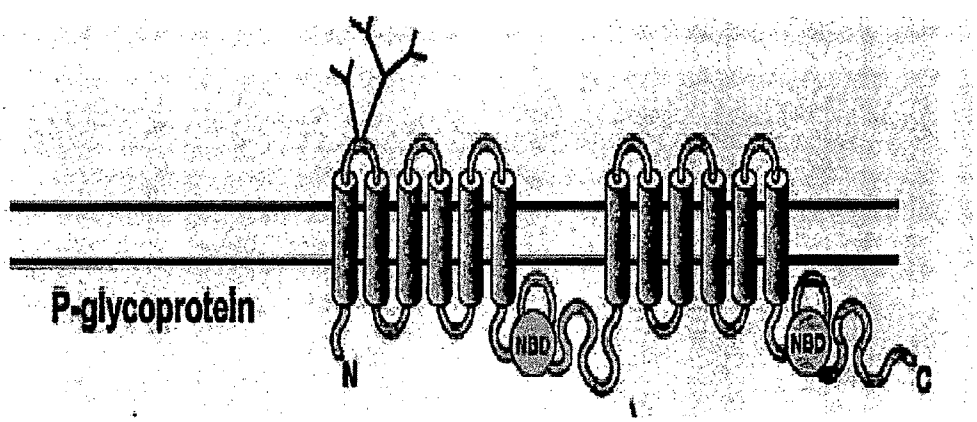


Figure 2.10: Predicted secondary structures of drug efflux transporters of the ATP-binding cassette family, P-glycoprotein consists of two transmembrane domains, each containing 6 transmembrane segments, and two nucleotide binding domains (NBDs). It is *N*-glycosylated (branches) at the first extracellular loop.

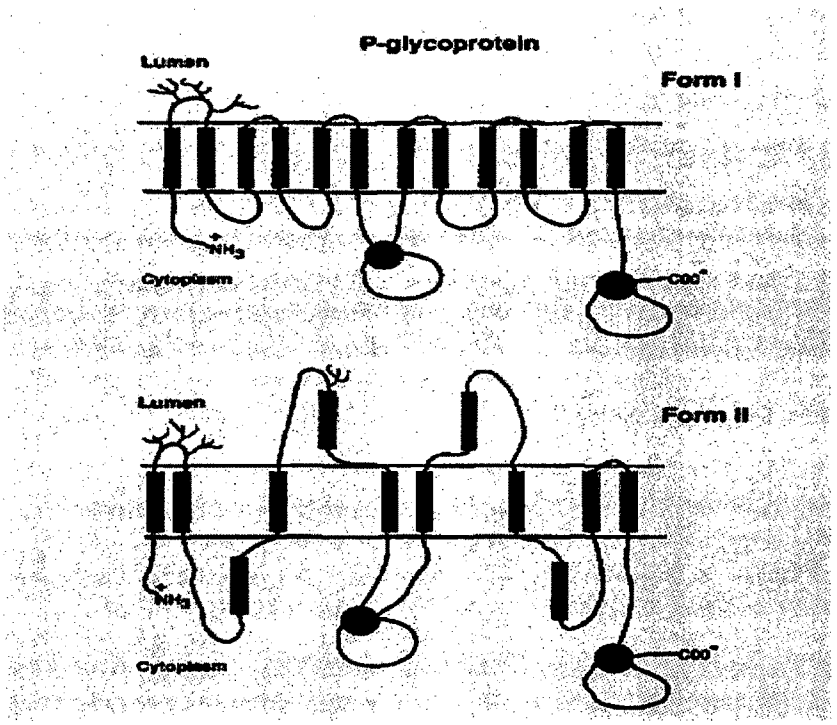


Figure 2.11: The proposed topological models for the MDRl gene product based on the hydropathy profile are presented at the top as form I. The alternative structure based on the evidence by Zhang JT and Ling V., 1995 is presented at the bottom as form 11. Solid bars represent transmembrane domains, while solid circles represent ATP-binding regions.

Although not supported by the electron microscopy, a growing body of evidence suggests that the P-glycoprotein may function as a dimer or oligomer. Freeze fracture analysis of multidrug resistant cell lines demonstrates an increase in the density of intramembrane particles with a diameter predicting a molecular mass of 340 kD (Sehested M et al., 1989). The particles cluster in the presence of verapamil, although not doxorubicin, suggesting they may represent localization of the P-glycoprotein. These results are complemented by radiation inactivation analysis of membrane preparations from multidrug resistance cell lines indicating that the P-glycoprotein has a molecular mass of 250 kD, about twice the size of the unglycosylated polypeptide (Boscoboinik D et al., 1990). Finally, immunoprecipitation of P-glycoprotein from multidrug resistant cells incubated with a crosslinking agent identifies a minor band at 340 kD in addition to the major 180 kD species (Naito M and Tsuruo T., 1992). The 340 kD band is

photolabelled with 3H-Azidopine, but only the 180 kD species is detected when the cells are treated with a reversible cross linking reagent. Finally, thermodynamic characterization of purified P-glycoprotein in defined lipid bilayers similarly suggest aggregation or oligomerization (Romsicki Y and Sharom SJ., 1997).These observations provide indirect evidence for a quaternary structure of the P-glycoprotein as a dimer in the membrane.

2.3.2 Mechanism of Pgp action

Various models were proposed to explain the mechanism of xenobiotic extrusion by Pgp; however, the exact site of substrate interaction with the protein is not well resolved. The three prevalent models namely pore model, flippase model and hydrophobic vacuum cleaner (HVC) model, explains the efflux mechanism to certain extent (Figure 2.10). Among these HVC model has gained wide acceptance in which P-gp recognizes substrates embedded in the inner leaflet of plasma membrane and transported through a protein channel (Higgings CF and Gottesman MM., 1992).

Rosenberg and co workers (Rosenberg MF et al., 2003) reported that three-dimensional conformation of P-gp changes upon binding of nucleotide to the intracellular nucleotide-binding domain. In the absence of nucleotide, the two transmembrane domains form a single barrel with a central pore that is open to the extracellular surface and spans much of the membrane depth, while upon binding nucleotides, the transmembrane domains reorganize into three compact domains that open the central pore along its length in a manner that could allow access of hydrophobic drugs directly from the lipid bilayer to the central pore of the transporter. ATP binding and hydrolysis was found to be essential for functioning of P-gp, where one molecule of drug is effluxed at the expense of two molecules of ATP (Gottesman MM and Pastan I., 1993).

Sauna and co workers (Sauna ZE et al., 2001) elucidated the catalytic cycle of P-gp, which expands the opportunity for the development of P-gp inhibitors, comprises of two cycles where drug and nucleotide binding sites coordinately function to efflux out the substrates by an ATP driven energy-dependent process. The drug and ATP initially binds to the protein at their own binding sites, where nucleotide hydrolyses to ADP yields energy for the extrusion of drug. The release of ADP from nucleotide binding site ends the first catalytic cycle followed by a conformational change that reduces affinity for both substrate and nucleotide. Further, the

second catalytic cycle starts by hydrolysis of another molecule of ATP and released energy is utilized to reorient the protein to its native conformation. Subsequent release of ADP completes another catalytic cycle, bringing P-gp molecule back to the original state, where it again binds to both substrate and nucleotide to initiate the next cycle.

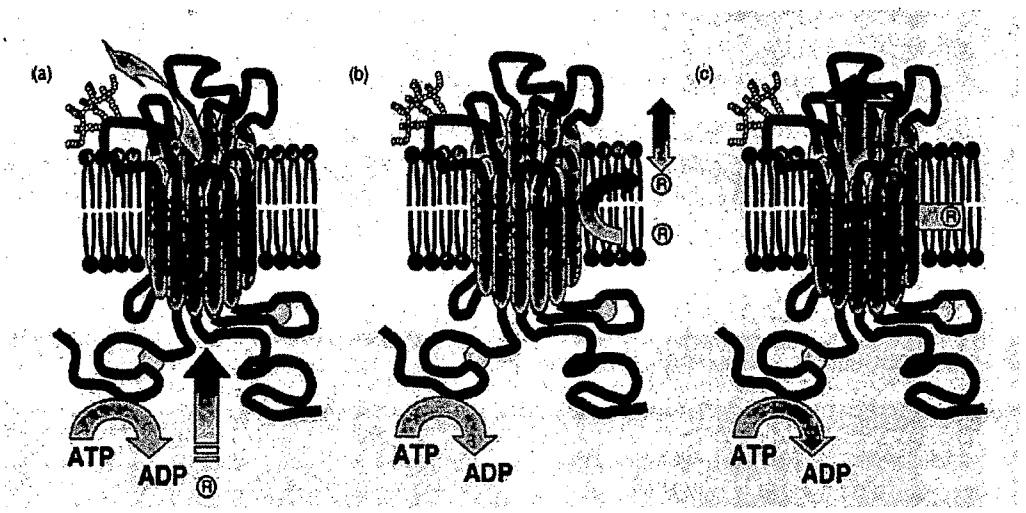


Fig. 2.12: Models proposed to explain the mechanism of drug efflux by P-gp. (a) Pore model, (b) flippase model and (c) hydrophobic vacuum cleaner model.

2.3.3 P- Glycoprotein inhibitors

Screening studies for P-gp–drug interactions identified a number of clinically important drugs as P-gp substrates, which are as diverse as anthracyclines (doxorubicin, daunorubicin), alkaloids (reserpine, vincristine, vinblastine), specific peptides (valinomycin, cyclosporine), steroid hormones (aldosterone, hydrocortisone) and local anaesthetics (dibucaine). Even dye molecules (Rhodamine 123) and pharmaceutical excipients exhibited P-gp substrate activity. Few of them were identified to inhibit P-gp, setting off an opportunity in MDR reversal. Improved clinical efficacy of various drugs observed by P-gp inhibition, especially drug subjected to MDR, lead to the design and development of modulators, which specifically block P-gp efflux and having improved toxicity profiles. P-gp inhibitors are gaining recognition to improve bioavailability by inhibiting P-gp in intestine, brain, liver and kidneys, which has been hypothesized and emphasized by many researchers in recent years (Van Asperen J et al, 1997).

Based on the specificity and affinity, P-gp inhibitors are classified to three generations. First-generation inhibitors are pharmacological actives, which are in clinical use for other indications but have been shown to inhibit P-gp. These include calcium channel blockers such as verapamil; immunosuppressants like cyclosporin A; anti-hypertensives, reserpine, quinidine and yohimbine; and antiestrogens like tamoxifen and toremifena. The usage of these compounds is limited by their toxicity due to the high serum concentrations achieved with the dose that is required to inhibit Pgp.

Second-generation modulators are agents that lack the pharmacological activity of the first-generation compounds and usually possess a higher P-gp affinity. However, inhibition of two or more ABC transporters leads to complicated drug–drug interactions by this class of compounds, which include non-immunosuppresive analogues of cyclosporin A, PSC 833; D-isomer of verapamil, dexverapamil; and others such as biricodar (VX-710), GF120918 and MS-209. On the other hand, several other novel third-generation P-gp blockers are under development, however, primarily with the purpose to improve the treatment of multidrug resistant tumours and to inhibit P-gp with high specificity and toxicity.

Modulators such as LY335979, OC144093 and XR9576 are identified to be highly potent and selective inhibitors of P-gp with a potency of about 10-fold more than the first and second-generation inhibitors In general, Pgp can be inhibited

(i) by blocking drug binding site either competitively, non-competitive or allosterically;
(ii) by interfering ATP hydrolysis (Shapiro AB and Ling V., 1997)
(iii) by altering integrity of cell membrane lipids (Drori S et al., 1995)

Although most of the drugs inhibit P-gp function by blocking drug binding sites, presence of multiple binding sites complicate understanding as well as hinder developing a true, conclusive SAR for substrates or inhibitors. However, the mode of handling of substrates and inhibitors are same by P-gp if the protein transport and/or inhibition are mediated only through binding sites. Then the issue to be addressed is how the substrates and inhibitors are discriminated at the molecular level. In this regard, and co workers (Eytan GD et al., 1996) proposed a plausible explanation that the modulator or inhibitor 'flipped' by P-gp can 'flop' back into the inner leaflet of the membrane, for further transport, which is very rapid creating a large difference between the rate of efflux of the substrate and inhibitor. Thus, the P-gp modulator is

cycled repeatedly, preventing efflux of substrates, which depends on the hydrophobicity of the compound. This concept had been proved from the drug delivery point of view that absorption of high affinity drugs to the protein need not necessarily be limited by P-gp, e.g. verapamil, if it is highly permeable whereas less permeable drugs though weak substrates may undergo a substantial extrusion mediated by P-gp, e.g. tanilol (Doppenschmitt S et al., 1999).

Compounds inhibiting ATP hydrolysis could serve as better inhibitors, since they are unlikely to be transported by P-gp, and these kind of agents will require at low dose which is well desirable to use locally at gut lumen. Quercitin, a naturally occurring flavanoid, has been proposed to block P-gp function by an unknown mechanism but in general by interfering ATPase activity. Commonly used pharmaceutical surfactants are emerging as a different class of P-gp inhibitors, which act by altering integrity of membrane lipids. The change in secondary and tertiary structure is found to be the reason for loss of P-gp function due to disturbance in hydrophobic environment by surfactants. In a series of studies by Hugger and co-workers (Hugger ED et al., 2002a; Hugger ED et al., 2002b), it was observed that the change in fluidity of cell membrane facilitates influx of P-gp substrates by surfactants like polyethylene glycol, cremophor EL and Tween 80, demonstrated in Caco-2 cell line. Surfactants seem to be better choice since they were already approved for routine use in pharmaceutical formulations. However, until now it has been tested at in vitro level, which should be further evaluated by animal or human studies. Likewise, the clinical reality of those P-gp inhibitors, interfering ATP hydrolysis and catalytic cycle will be hampered by lack of understanding of the exact mode of inhibition which remains as a potential area for further research. Wang and co workers used Paclitaxel as the Pgp substrate and screened the Pgp inhibiting ability of a series of surfactants on NIH 3T3 and HCT-8 cell lines. A few are tabulated in table 2.11.

Pharmacological category	Examples
Antiarrhythmics	Amioderone, lidocaine, quinidine
Antibiotics and antifungals	Cefoperazone, ceftriazone, erythromycin, itraconazole, ketoconozole, aureobasidin A
Antimalarials and antiparasites	Chloroquine, emetine, hydroxychloroquine, quinacrine, quinine
Calcium channel blockers	Bepridil, diltiazem, felodipine, nifedipine, nisoldipine, nitrendipine, tiapamil, verapamil
	Calmodulin antagonist Chlorpromazine, trifluperazine
Cancer chemotherapeutics	Actinomycin D, colchicines, daunorubicin, doxorubicin, etoposide, mitomycin C, mithramycin, podophyllotoxin, puromycin, taxol, topotecan, triamterene, vinblastine, vincristine
Fluorescent dyes	BCECF-AM, Fluro-2, Fura-2, Rhodamine 123, Hoechst 33342
HIV protease inhibitors	Indinavir, nelfinavir, ritonavir, saquinavir
Hormones	Aldosterone, clomiphene, cortisol, deoxycorticosterone, dexamethosone, predinisone, progesterone analogs, tamoxifen, hydrocortisone, testosterone
Immunosuppressants	Cyclosporin A, cyclosporin H, tacrolimus, sirolimus
Indole alkaloids	Reserpine, yohimbine
Local anaesthetics	Bupivacaine
Surfactants/solvents	Cremophor-EL, triton X-100, Tween 80
Toxic peptides	*N*-Acetyl-leucyl-leucinal, gramicidine D, valinomycin
Tricyclic antidepressants	Desipramine, trazadone
Miscellaneous	Components of grape and citrus fruit juice, ethidium bromide, ivermectin, quercetin

Table 2.9: Agents that interact with P-glycoprotein

P-glycoprotein substrates	P-glycoprotein modulator	Experimental model	Pharmacokinetic effect
Digoxin	Verapamil	Single-pass perfusion in rats	Increase in absorption rate
Doxorubicin	Cyclosporine	Cancer patients	Inhibitor dose-dependent permeability enhancement
Doxorubicin	PSC 833	Cancer patients	~ 50% increase in AUC
Paclitaxel	R-verapamil	Cancer patients	Delayed mean paclitaxel clearance and increased peak Concentration
Paclitaxel	GF120918	mdr1ab(−/−) knockout mice and wild-type mice	Enhanced BA
Paclitaxel	VX-710	Cancer patients	More than 50% decrease in paclitaxel clearance
Paclitaxel	MS-209	Rats and mice	1.9- and 4.5-fold increase in BA in rats and mice, respectively
Doxorubicin	LY335979	Cancer patients	~ 25% increase in BA at doxorubicin dose of 60 mg/m^2 and ~ 15% increase at a dose of 75 mg/m^2
Paclitaxel	OC144093	Cancer patients	~ 1.5-fold increase in AUC
Docetaxel	R101933	Cancer patients	Pharmacokinetics did not alter in the presence of inhibitor but the faecal excretion of docetaxel decreased significantly
Doxorubicin	XR9576	Cancer patients	44% increase in AUC

AUC – Area under the curve; BA – Bioavailability

Table 2.10: Examples for improved pharmacokinetics of P-gp substrates with co-administration of P-gp inhibitors

Excipient	Tradename
d-α-tocopheryl polyethylene glycol 1000 succinate	Vitamin E TPGS
Diethylene glycol monoethyl ether	Transcutol P
PEG-30 stearate	MYRJ 51 NENA
PEG-32 lauric glycerides	Gelucire 44/14
PEG-35 castor oil	CREMOPHOR EL
PEG-40 hydrogenated castor oil	CREMOPHOR RH 40
PEG-8 caprylic/capric glycerides	Labrasol
Poloxamer 124	Pluronic L44
Poloxamer 188	Pluronic F68
Poloxamer 235	PLURONIC P85
Poloxamer 237	Pluronic F87
Poloxamer 338	Pluronic F108
Poloxamer 407	Pluronic F127
Polyoxyethylene 20 Sorbitan Monolaurate	Polysorbate 20
Polyoxyethylene 20 Sorbitan Monopalmitate	Polysorbate 40
Polyoxyethylene 20 Sorbitan Monostearate	Polysorbate 60
Polyoxyethylene 20 Sorbitan Monooleate	Polysorbate 80
Steareth-100	BRIJ 700

Table 2.11: Excipients Used in Oral, IV, and IM Formulations and Screened With NIH/ 3T3 and HCT-8 Cells (Wang SW et al., 2004)

Role of P-Glycoprotein in brain uptake

Drug Efflux Transporters in the CNS

For drugs that directly act on targets in the central nervous system (CNS), sufficient drug delivery into the brain is a prerequisite for CNS action. However, drug delivery to the brain is often hindered by two barrier systems, namely, the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier (BCSFB). The BBB consists of capillary endothelial cells with tight junctions that lack small aqueous pores. Astrocytes, pericytes, and the extracellular matrix components are believed to control the integrity of the BBB (Brightman MW., 1988). In human adults, the vascular capillaries account for about 1% of the brain volume and the surface area of capillary endocellular cells is estimated to be approximately 10m2. On the other hand, the BCSFB that prevents the free passage of substrates between blood and CSF is made up by the epithelium of the choroid plexus. In the BCSFB, the tight junctions are between the epithelial cells, not the endothelial cells (Johanson CE., 1988).

The functional role of the BBB and BCSFB is to protect the brain against toxic xenobiotics by separating the brain from the cerebral blood circulation. As a result, only lipophilic xenobiotics and drugs can cross the barriers and enter the brain by way of passive diffusion. Studies by many investigators have shown that there is a strong positive correlation between lipophilicity and brain penetration of drugs (Pardridge WM., 1981; Rapport SI., 1976). Although lipophilicity is an important factor in determining the brain penetration of drugs, many lipophilic drugs exhibit poor brain penetration. In a rat study, Levin (Levin VA., 1980) reported a good correlation between the in vivo brain penetration of 22 compounds and their lipophilicity. However, they found that vincristine and epipodophylotoxin displayed poor brain penetration, despite relatively high lipophilicity (log P value of 2.8). In addition, many other lipophilic compounds also exhibit poor brain penetration. For example, although a potent CCKB receptor antagonist candidate is a lipophilic compound with a log P value of 3.6, it exhibits poor brain penetration (Lin TH and Lin JH., 1990). These results suggest that factors other than lipophilicity may also play an important role in the transport of drugs across the brain. It has been suggested that hydrogen bonding may also influence brain penetration.

A negative correlation was found between the brain penetration of lipophilic compounds and the total number of hydrogen bonds; the greater the number of hydrogen bonds, the lower the permeability (Chikhale EG et al., 1994; Pardridge WM., 1981). In addition to lipophilicity and

number of hydrogen bonds, brain penetration studies in animals have revealed that the molecular size of drugs is also an important determinant for brain penetration (Levin VA., 1980). However, the poor brain penetration of some lipophilic drugs still cannot be explained by factors such as hydrogen bonds and molecular size alone. Without knowing an exact cause, these compounds were regarded as ''outlier compounds'' in regards to brain penetration. No connection was made between the possible efflux function of P-gp in the BBB and the poor brain penetration of these lipophilic compounds observed in animals until the findings of P-gp in brain capillaries by Thiebaut and co workers (Thiebaut F et al. 1989). Using monoclonal antibodies, they demonstrated that P-gp was highly expressed on the apical surface of the endothelial cells of the brain capillaries. Recognizing these findings, it is now clear that the observed poor brain penetration of some lipophilic drugs is due mainly to the efflux transport of P-gp. Since the first discovery of brain P-gp by Thiebaut et al. in 1989 (Thiebaut F et al., 1989), numerous additional studies confirmed that P-gp is expressed in the endothelium of brain capillaries of several species. Jette and co workers (Jette L et al., 1993) showed that P-gp was expressed in capillaries isolated from human, bovine, and rat brain.

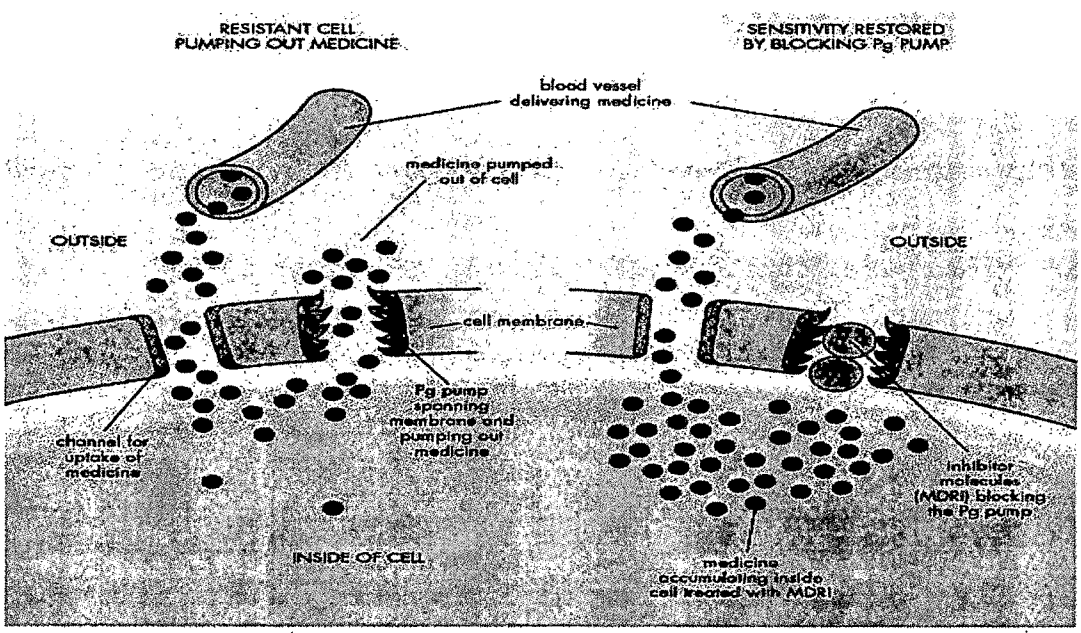
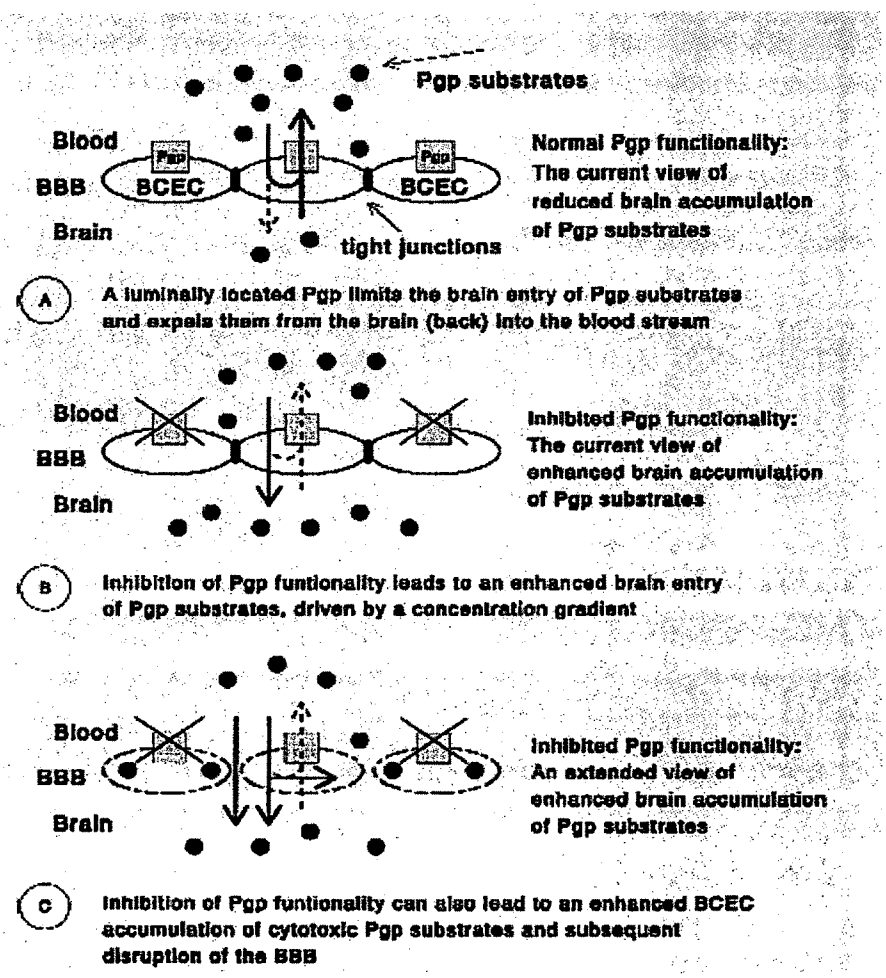


Figure 2.13: Pgp activity at the cell membrane of resistant cells and resistant cells treated with Pgp pump blockers



Pgp – P-Glycoprotein; BBB – Blood Brain Barrier; BCEC - Brain Capillary endothelial cells

Figure 2.14: A - Schematic drawing of the role of Pgp in the BBB.
B – Effect of Pgp inhibition
C – Simultaneous exposure to Pgp substrates

Similarly, the expression of P-gp in capillaries of bovine brain was reported by Tsuji and co workers (Tsuji A et al., 1992). In addition to rat, bovine, and human brain, P-gp was detected in the brain of mice and pigs (Hegmann EJ et al., 1992). Recent studies suggest that P-gp is also expressed in the choroid plexus (Kusuhara H and Sugiyama Y., 2001a; Kusuhara H and Sugiyama Y., 2001b). The choroid plexus lines the lateral ventricles and the roof of the third and fourth ventricles. Staining human and rat choroid plexus with MRK16, Rao and co

workers (Rao VV et al., 1999) showed a subapical expression pattern of Pgp confined to the epithelial cells of choroid plexus. In this study, they also showed a net directional (basolateral to apical) transport of P-gp substrates ([3H] taxol and ^{99m}Tc-sestamibi) across confluent monolayers of epithelial cells of rat choroid plexus. The directional transport of both [3H] taxol and ^{99m}Tc-sestamibi was diminished on addition of GF120918, a potent Pgp inhibitor. These results suggest the choroid plexus as a potentially important site for selective delivery of Pgp substrates from blood into the brain.

Because of the anatomical localization, P-gp acts as an efflux transporter in the BBB by removing drugs out of the brain into cerebral blood circulation, but it functions as an influx transporter in the choroid plexus by transporting drugs into the brain from the circulation. Because of structural and anatomic differences, the importance of the role of the choroid plexus in brain uptake has been questioned. Some investigators believe that the choroid plexus has a relatively minor role in drug transport in comparison with the substantially more extensive brain capillaries. The main argument has been that the surface area of the choroid plexus is much smaller than the total surface area of brain capillaries. It has been suggested that the surface area of the capillaries perfusing regions of choroid plexuses is approximately 5000-fold less than the surface area of brain capillaries (Pardridge WM et al., 1981). However, this view that the surface area of choroid plexus is much smaller than that of BBB has been questioned. In the rat, the total surface area of the choroid plexuses and brain capillaries was estimated to be 75 and 155 cm^2, respectively (Keep RF and Jones HC., 1990). This value of surface area of choroid plexuses is much greater than generally believed. However, it is of interest to note that the importance of the role of P-gp influx in the choroid plexuses is apparently not as important as in the BBB, even though the surface area of choroid plexus is comparable to that of BBB.

If the role of P-gp influx in the choroid plexuses were more important than the P-gp efflux in the BBB, deficiency of P-gp would result in a decrease in brain concentrations of P-gp substrates, rather than an increase in brain drug concentration. However, this appears not to be the case. As will be discussed below, deficiency of P-gp in the mdr1a (-/-) mice always exhibit a much better brain penetration for P-gp substrates than wild-type mice. More kinetic studies are required to fully understand the quantitative role of the P-gp influx in the choroid plexuses in brain penetration of P-gp substrates. Although the expression of multidrug resistance-

associated protein (MRP) has been observed in various in vitro cultured brain endothelial cells, the expression of MRP at the BBB in vivo is still in question. No MRP1 expression has been detected in freshly isolated human brain capillaries, whereas MRP1 expression has been detected by immunochemistry in cultured human endothelial cells derived from the same isolated human brain capillaries (Seetharaman S et al., 1998). It has been speculated that the expression of MRP1 may be up-regulated during cell culture. Similarly, up-regulation of Mrp1 was observed in rat cultured endothelial cells. Expression of Mrp1 was much lower in the freshly isolated rat brain capillaries than in cultured endothelial cells derived from these isolated rat brain capillaries (Regina et al., 1998). The Mrp1 protein has been found on the basolateral side of the epithelial cells of rat choroid plexus (Rao VV et al., 1999). Because of its localization, MRP1 can act as an efflux transporter by removing drugs from the CSF to blood circulation. In contrast to the BBB, high expression of Mrp1 protein was observed in the rat choroid plexus (Nishino J et al., 1999). Western blot analysis indicated that expression of Mrp1 protein in the choroid plexus was 4–5 times higher than in the lung, one of the tissues in which Mrp1 is highly expressed.

Involvement of P-Glycoprotein in Brain Uptake

Direct evidence for the involvement of P-gp in brain uptake of drugs is mainly derived from animal studies, particularly with the transgenic animal model of P-gp deficient mdr1a (-/-) mice. Following intravenous administration of [3H] ivermectin, radioactivity level in the brain of mdr1a (-/-) was 83-fold higher than that of wild-type (+/+) mice, whereas the differences in radioactivity levels between these two groups of mice were less than threefold for other tissues, including liver, kidney, small intestine, and plasma (Schinkel AH et al., 1994).

In another study, when [3H]digoxin and [3H]cyclosporine A were given intravenously, considerably higher brain levels of radioactivity (17- and 55-fold, respectively) were observed in mdr1a (-/-) mice compared with mdr1a (+/+) mice, whereas only a moderate increase in radioactivity levels (two- to threefold) of these two drugs was observed for liver, kidney, small intestine, and plasma of mdr1a (-/-) mice (Schinkel AH et al., 1995). Similarly, marked differences in brain concentration of P-gp substrates were also observed between mdr1a/1b (-/-) double knockout and wild-type mice (Schinkel AH et al., 1997). A 27-fold increase in the brain concentration of digoxin was observed in mdr1a/1b (-/-) double knockout mice compared to the wild-type mice, whereas only a 2.5-fold increase in digoxin concentration was seen in

the liver, kidney and plasma of the double knockout mice. The P-gp-deficient mdr1a (-/-) mice have also been used to assess the role of P-gp in brain uptake of numerous P-gp substrates, including HIV protease inhibitors (amprenavir, nelfinavir, indinavir, and saquinavir), anticancer drugs (taxol, vinblastine), quinidine, tacrolimus, loperamide, and verapamil (Choo EF et al., 2000; Kim RB et al., 1998; Kusuhara H et al., 1999; Polli JW et al., 1999). For these drugs, marked differences in brain concentrations were also observed between mdr1a (-/-) and mdr1a (+/+) mice, whereas only small differences were seen in drug concentration in other tissues. Following oral administration of [14C]-amprenavir, the mean radioactivity level in the brain of mdr1a/1b (-/-) double knockout mice was 27-fold greater than that in wild-type mice (Polli JW et al., 1999).

Similarly, the radioactivity level in the brain of mdr1a (-/-) mice was 28-fold higher than that in mdr1a (+/+) mice after an oral dose of [14C]-nelfinavir (Choo EF et al., 2000). The ratio of brain concentration of quinidine in mdr1a (-/-) mice to that in mdr1a (+/+) mice was estimated to be 29 following intravenous administration (Kusuhara H et al., 2001). Similarly, the ratio of brain concentration of tacrolimus in mdr1a (-/-) mice to that in mdr1a (+/+) mice was estimated to be 33 after an oral dose (Yokogawa K et al., 1999). Collectively, these results strongly suggest that P-gp plays a quantitatively important role in brain uptake of drugs. As discussed above, the low expression of MRP1/Mrp1 in the freshly isolated brain capillaries suggests that the role of this efflux transporter at the BBB is limited. The limited role of Mrp1 at the BBB was further supported by recent studies in Mrp1 (-/-) mice (Cisternino S et al., 2003). There was no significant difference in BBB transport of 17b-estradiol-D-17b-glucuronide (E217bG), a substrate of Mrp1 transporter, between wild-type (0.16_0.02 mL/s/g) and Mrp1 deficient (-/-) mice (0.14_0.02 mL/s/g) during a 120-sec brain perfusion. In contrast to the BBB, the Mrp1 protein is highly expressed on the basolateral side of the epithelial cells of rat choroid plexus, suggesting that MRP may play a significant role in brain uptake of drugs that are MRP substrates via efflux transport at the BCSFB (Rao VV et al., 1999).

Following intracerebroventricular administration to rats, the elimination of E217bG from CSF was very rapid, and < 2% of the dose remained in the CSF 20 min after dosing (Nishino et al., 1999). In a recent study with Mdr1a/Mdr1b double knockout mice and Mrp1/Mdr1a/Mdr1b triple knockout mice, Wijnholds J et al. (2000) have demonstrated that although Mrp1 plays a minor role at the BBB, it plays a quantitatively important role at the BCSFB.

Following intravenous administration of etoposide (a substrate for both P-gp and Mrp1), there was no significant difference in brain concentration of etoposide between the double knockout and triple knockout mice. These results indicated that Mrp1 played a minor role of etoposide uptake at the BBB. In contrast, the CSF concentration of etoposide in the triple knockout mice was eightfold higher than in double knockout mice, suggesting a significant role of Mrp1 at the BSCFB. Evidence for P-gp involvement for brain uptake in animals is easy to obtain by direct sampling of brain tissues. Because of the inaccessibility of brain tissue, direct evidence of P-gp role in brain uptake of drugs in humans is difficult to obtain. Therefore, the evidence of P-gp involvement in brain uptake in humans is often obtained indirectly from clinical studies. For example, loperamide (a potent opiate used as an antidiarrheal drug) produced no respiratory depression in patients when administered alone, but respiratory depression occurred when loperamide was given with quinidine (Sadeque AJM et al., 2000). Because both loperamide and quinidine are P-gp substrates, it is believed that the observed respiratory depression is due to P-gp-mediated inhibition resulting in increased delivery of loperamide to the brain. The involvement of P-gp in brain uptake of loperamide is further supported by animal studies. Loperamide produced opiate-like effects in Mdr1a (-/-) mice but not in wild-type Mdr1a (þ/þ) mice (Schinkel et al., 1996). Tissue distribution study revealed that the brain concentration of loperamide in Mdr1a (-/-) mice was about 14-fold higher than in wild-type Mdr1a (+/+) mice 4 hr after oral administration of loperamide. Like this example, the role of P-gp in limiting the brain penetration of loperamide in humans can only be unequivocally proven in conjunction with animal studies. Recently, the positron-emission tomography (PET) technique has been used as a noninvasive method for investigating the role of P-glycoprotein in brain uptake of drugs in humans. Although PET scanning has been used for studying the effect of P-gp on brain uptake of drugs in animals for a while, its use for investigating the Pgp's involvement in brain uptake of drugs in humans has only been very recent.

P-Glycoprotein Plays a Very Important Role in Brain Uptake

From the studies described above, it is evident that P-gp plays a very important role in limiting brain uptake of P-gp substrates. The importance of the role of P-gp in brain uptake is further supported by interaction studies mediated by P-gp inhibition. For instance, pretreatment of mdr1a (+/+) mice with LY-335979 (25 mg/kg, i.v.) resulted in a 37-fold increase in brain concentration of nelfinavir but had little effect on plasma concentrations (Choo et al., 2000). Similarly, pretreatment with intravenous valspodar (PSC833, 25 mg/kg i.v.) resulted in an 80-

fold increase in brain concentration of nelfinavir in mdr1a (+/+) mice (Choo et al., 2000). On the other hand, LY-335979 and valspodar had little effect on both the brain and plasma concentration of nelfinavir in P-gp deficient mdr1a (-/-) mice. Both LY-335979 and valspodar are specific and potent P-gp inhibitors, which inhibit digoxin transport with an IC50 value of 0.024 and 0.11 mM, respectively (Choo et al., 2000). In another study, a 10-fold increase in brain concentration of digoxin was observed when valspodar (50 mg/kg) was given orally to mdr1a (+/+) mice (Mayer U et al., 1997). Similar observations were also reported for another potent P-gp inhibitor, GF-120918. Pretreatment of mdr1a (+/+) mice with GF-120918 (250 mg/kg/day for 4 days, orally) led to a 13-fold increase in brain concentrations but resulted in only a modest increase (twofold) in plasma concentrations of amprenavir (Polli et al., 1999). It is still a puzzle as to why the P-gp plays such a more important role in limiting drug uptake at the BBB compared to its role in absorption of drugs from the small intestine in which P-gp is also highly expressed. The rate of brain penetration (dA_{brain}/dt) is the net difference between the rate of influx process (dA_{inf}/dt) by simple diffusion and the rate of the P-gp efflux process

$$dA_{brain}/dt = dA_{inf}/dt - dA_{eff}/dt$$

The marked inhibitory effect of P-gp on brain penetration suggests that for most P-gp substrates the rate of influx process by simple diffusion at the BBB is smaller than the rate of P-gp efflux process. There are many reasons as to why the rate of influx process could be smaller than (or equal to) the rate of efflux process. In contrast to the very high drug concentration in the intestinal lumen during absorption, drug concentrations in cerebral blood circulation are relatively low.

2.4 POLY LACTIDE CO-GLYCOLIDE (PLGA) MICROSPHERES AS DRUG DELIVERY SYSTEMS

Introduction to Lactide copolymers

Polylactide is the most frequently used polyester in biomedical applications due to its many favorable characteristics, e.g., high strength and biocompatibility. Environmental concern has led to escalated interest in using Polylactide and other biodegradable polymers as an alternative to traditional commodity plastics. PLA is synthesized either from lactic acid by a condensation reaction or more commonly by ring-opening polymerization of cyclic lactide monomer. Polymers derived from lactic acid by polycondensation (PC) are generally referred to as poly (lactic acid) and the ones prepared from lactide by ring-opening polymerization (ROP) as poly (lactide). Both types are generally referred to as PLA. The selection of polymer manufacturer is critical in determining the performance of the designed drug delivery system because the poly lactic acid (PLA), poly glycolic acid (PGA) and poly lactide co-glycolide (PLGA) differ in properties if there is a change in the manufacturing process or catalyst used in the polymerization process. Purac, Birmingham Polymers, Boehringer Ingelheim, Sigma Chemical Company and Poly Science are some leading suppliers of GMP grade lactide polymers

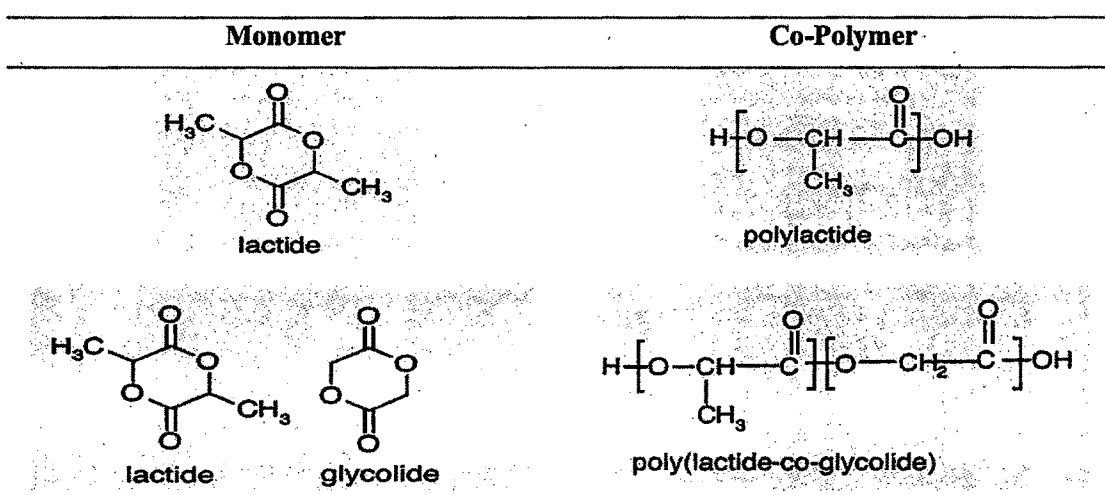


Figure 2.15: Structure of some common biodegradable polymers and their monomers of pharmaceutical interest

Polymer type	Inherent viscosity (dL/g)	Melting point (°C)	Glass transition temperature (°C)	Solubility	Specific gravity (g/mL)	Tensile strength (psi)	Elongation (%)	Modulus (psi)
50/50 DL-PLG	0.55-0.75	Amorphous	45-50	1,2,3,4,5,6	1.34	6000-8000	3-10	$2\text{-}4 \times 10^5$
65/35 DL-PLG	0.55-0.75	Amorphous	45-50	1,2,3,4,5,6	1.30	6000-8000	3-10	$2\text{-}4 \times 10^5$
75/25 DL-PLG	0.55-0.75	Amorphous	50-55	1,2,3,4,5,6	1.30	6000-8000	3-10	$2\text{-}4 \times 10^5$
85/15 DL-PLG	0.55-0.75	Amorphous	50-55	1,2,3,4,5,6	1.27	6000-8000	3-10	$2\text{-}4 \times 10^5$
DL-PLA	0.55-0.75	Amorphous	55-60	1,2,3,4,5,6	1.25	4000-6000	3-10	$2\text{-}4 \times 10^5$
L-PLA	0.90-1.20	173-178	60-65	1,4,5	1.24	8000-12000	5-10	$4\text{-}6 \times 10^5$
PGA	1.40-1.80	225-230	35-40	5	1.53	10000+	15-20	1×10^6
PCL	1.00-1.30	58-63	55-60	1,4,5,6	1.11	3000-5000	300-500	$3\text{-}5 \times 10^4$

1 = methylene chloride; 2 = tetrahydronfuran; 3 = ethyl acetate; 4 = chloroform; 5 = hexafluroisopropanol; 6 = acetone

DL-PLG = poly (DL-lactide-co-glycolide); DL-PLA = poly (DL-lactide); L-PLA = poly (L-lactide); PGA = poly (glycolide);

PCL = poly (ε - caprolactone)

Table 2.11: Physical and chemical properties of some commonly used lactide polymers

The crystal structure of PLA was reported to be a left-handed helix conformation for the α-form (de Santis P et al., 1968). The melting temperature and degree of crystallinity are dependent on the molar mass, thermal history, and purity of the polymer (Jamshidi K et al., 1988; Migliaresi C et al., 1991; Migliaresi C et al., 1991), and the crystallization kinetics and melting behavior of poly (lactide) of different optical purity have been investigated in several studies (Vasanthakumari R and Pennings AJ., 1983; Marega C et al., 1992; Mazzullo S et al., 1992). It has been observed that an optical purity of at least 72-75%, corresponding to about 30 isotactic lactyl units, is required for the crystallization to take place (Tsuji H and Ikada Y., 1992). Sarasua and co workers (Sarasua JR et al., 1998) were able to crystallize a poly (lactide) of as low as 43% optical purity, when polymerizing by using Salen – Al - OCH3 (a complex resulting when reacting a Schiff base on $AlEt_2Cl$) as initiator, which was explained by the preference for the formation of long isotactic sequences. By utilizing stereoselective catalysts in the polymerization, semicrystalline polymers have been prepared both from meso-lactide (Ovitt TM and Coates GW., 1999) and rac-lactide (Radano CP et al., 2000; Ovitt TM and Coates GW., 2000).

Stability

Thermal stability

The thermal stability of aliphatic polyesters is in general limited (Gupta MC and Deshmukh VG., 1982a; Gupta MC and Deshmukh VG., 1982b; Zhang X et al., 1992). The thermal stability of lactic acid based polymers is accordingly poor at elevated temperatures, but the reported studies are mainly concerning poly (l-lactic acid), poly (l-lactide), and poly (rac-lactide). Gupta and Deshmukh (Gupta MC and Deshmukh VG., 1982a) concluded that the carbonyl carbon - oxygen linkage is the most likely one to split by isothermal heating. This postulate is based on the detected changes in the electron charge distribution of the nonbonding electrons of the carbonyl carbon-oxygen linkage for the thermally degraded poly (lactide). Any noticeable change was not found for the carbon-oxygen bond. A significantly larger amount of carboxylic acid end groups than hydroxyl end groups was identified, which indicated a break of the carbonyl carbon-oxygen linkage. The kinetics for the thermal degradation of poly (lactide) was suggested to be of first order (Gupta MC and Deshmukh VG., 1982b). Reactions involved in the thermal degradation of lactic acid based polymers can be thermohydrolysis (Soedergaerd A and Naesman JH., 1994), zipper-like depolymerization (Soedergaerd A and Naesman JH., 1994; Zhang X. et al., 1992), thermooxidative degradation (McNeill IC and

Leiper HA., 1985), and transesterification reactions (Tighe BJ., 1984; Garozzo D et al., 1986; Kopinke FD et al., 1996).

Radiation stability

The irradiation effects on aliphatic polyesters were studied by D'Alelio and co workers (D'Alelio GF et al., 1968) in the late 1960s. The irradiation effects were evaluated by using intrinsic viscosity measurements, and it was concluded that the cross-linking to scission ratio for aliphatic polyesters increased upon irradiation as a function of increasing -CH2- to –COO- ratios in the main chain. The radiation stability of poly (l-lactide) (Collet JH et al., 1989) and racemic copolymers of l-lactide and d-lactide has been reported (Birkinshaw C et al., 1992). The stability of copolymers of lactide and glycolide (Collet JH et al., 1989) as well as lactide and 1-caprolactone have also been studied, but as the information on the radiation stability of copolymers of lactide and other ring-formed monomers is limited, has also the radiation stability of homopolymers been reviewed. Poly (lactide) mainly undergoes chain-scissions at doses below 250 kGy (Collet JH et al., 1989; Gupta MC and Deshmukh VG., 1983). For higher doses, crosslinking reactions increase as a function of the irradiation dose both in air and in inert atmosphere (Gupta MC and Deshmukh VG., 1983). The irradiation has been suggested to cause reactions in the amorphous phase of the polymer (Collet JH et al., 1989) and a difference in the radiation effects may therefore be expected for poly (rac-lactide) and poly (l-lactide). The irradiation of poly (glycolide) has been reported to cause a decrease in the molar mass (Gilding DK and Reed AM., 1979), but it has also been suggested that it causes about the same extent of cross-linking as chain-scission (Pittman Jr. CU et al., 1978). The radiation was suggested to cause an abstraction of hydrogen from the methylene group resulting in radical formation at this carbon atom or by loss of the esters linkage by chain-scission [308]. The radiation has been reported to reduce the tensile strength and speed up the start of the hydrolytic degradation for PGA fibers (Chu CC., 1985).

Hydrolytic stability

Hydrolysis of polymers leads to molecular fragmentation, which can be regarded as a reverse polycondensation (Williams DF., 1989). This process can be affected by various factors such as chemical structure, molar mass and its distribution, purity, morphology, shape of the specimen and history of the polymer, as well as the conditions under which the hydrolysis is conducted (Vert M et al., 1991). The hydrolytic degradation of lactic acid based polymers is a

phenomenon, which is undesired at certain circumstances, e.g. during processing or material storage, but beneficial in other applications, for example in medical devices or compostable packages. The hydrolysis of aliphatic polyesters starts with a water uptake phase followed by hydrolytic splitting of the ester bonds in a random way according to the Flory principle, which postulates that all linkages have the same reactivity. This was demonstrated by Shih (Shih C., 1995), who reported on random scission during alkalic hydrolysis of poly (rac-lactide) when acid catalyzed hydrolysis gives faster chainend scissions (Shih C., 1995). The later phenomenon can be explained by a growing amount of chain ends, which with time leads to an increased probability of breaks at the chain ends. The initial degree of crystallinity of the polyesters affects the rate of hydrolytic degradation as the crystal segments reduce the water permeation in the matrix.

The amorphous parts of the polyesters have been noticed to undergo hydrolysis before the crystalline regions because of a higher rate of water uptake. The first stage of the hydrolytic degradation is accordingly located to the amorphous regions where the molecule fragments, that are tying the crystal blocks together by entanglement, are hydrolyzed. The remaining undegraded chain segments therefore obtain more space and mobility, which lead to reorganizations of the polymer chains and an increased crystallinity (Fischer EW et al., 1973). These phenomena could in the study by Chu (Chu CC., 1985) optically be noticed by whitening specimens, caused by the degradation induced molecular reorganizations with a simultaneous decrease in the mechanical strength and molar mass. The temperature during the hydrolysis is of major importance for the degradation rate. This is because of an increased hydrolysis rate at elevated temperatures (Gilding DK and Reed AM., 1979), but also a result of the increased flexibility of the polymer when the temperature is above the glass transition temperature of the polymer.

The hydrolytic degradation for poly (l-lactide) of different molar mass as well as the hydrolytic degradation of high molar mass poly (ester-urethanes) prepared from lactic acid (Hiltunen K et al., 1988) have been reported. The hydrolytic degradation of poly (lactide) homo- and copolymers is homogeneous, i.e. the number average molar mass has significantly decreased before any weight loss can be noticed. In the second stage of the hydrolysis the hydrolytic degradation of the crystalline regions of the polyester leads to an increased rate of mass loss and finally to complete resorbtion (Li SM et al., 1990). The degradation of poly (lactide) in

aqueous medium was reported by Li and coworkers (Li SM et al., 1990) to proceed more rapidly in the center of a specimen. The explanation to this behavior was an autocatalytic effect due to the increasing amount of compounds containing carboxylic end groups. These low molar mass compounds were not able to permeate the outer shell. The degradation products in the surface layer were in contradiction continuously dissolved in the surrounding buffer solution (Li SM et al., 1990). The reasons for the differences in the stability can be found in the purity of the polymer, molar mass and its distribution, crystallinity, and orientation (Joziasse CAP et al., 1998). A recent study has also shown that the type of initiator/catalyst system significantly affects the water uptake and hydrolytic degradation rate, which was explained by the differences in the hydrophobicity of the initiators/catalysts used (Schwach G and Vert M., 1999).

Stability in biological environment

Biodegradation has been defined as "the gradual breakdown of material mediated by specific biological activity". This process may be initiated and maintained by enzymes or microorganisms and include abiotic reactions like hydrolysis and/or oxidation, which result in a fragmentation of the molecules. The biodegradation of lactic acid based polymers for medical applications has been investigated in a number of studies in vivo (Schwach G and Vert M., 1999; Pistner H et al., 1993; Pistner H et al., 1994) and some reports can also be found on the degradation in other biological systems (Hakkarainen M et al., 2000). Williams (Williams DF., 1981) reported on a screening study, where the degradation of PLLA in presence of a number of different enzymes was studied. It was concluded that pronase, proteinase K, and bromelain had a significant effect on the hydrolysis rate and that ficin, esterase, and trypsin at a smaller extent (Williams DF., 1981). Torres and co workers (Torres A. et al, 1996) reported on a study where the ability of certain microorganisms to use lactic acid based polymers as carbon sources. Fusarium moniliforme and Penicillium roqueforti were able to assimilate hydrolyzed polymers of rac-lactide and one strain of Fusarium moniliforme copolymers of rac-lactide and glycolide.

A significant increase in the degradation rate was noticed for poly (l-lactide) during degradation in the presence of mixed culture of microorganisms compared to abiotic degradation (Hakkarainen M et al., 2000). The effect of molar mass of poly (l-lactic acid), ranging from 0.26 to 2.88 kDa, on the biodegradation has been studied by Karjomaa and co

workers (Karjomaa S et al., 1998). The degradation was found to decrease with increasing chain length and proceeded more rapidly in biotic environment (Karjomaa S et al., 1998). The effect of the stereo structure of poly (rac-lactide) on the hydrolytic degradation in presence of proteinase K was reported by Tsuji and Miyauchi (Tsuji H and Miyauchi S., 2001). The presence of longer d-lactyl unit sequences than 10 and an l-lactyl content below 0.3 will reduce the enzymatic degradability of the stereo copolymer. The effects of physical ageing and morphology on the enzymatic degradation of poly (l-lactic acid) were studied by McCarthy and coworkers (Cai H et al., 1996). It was concluded that morphological changes due to the ageing affect the rate of degradation by reducing the mobility of the polymer chains, which was reflected in a lower degradation rate (Cai H et al., 1996).

Drug release mechanisms

Mathematical drug release modeling of bioerodible delivery systems are not as advanced as the modeling of diffusion or swelling controlled devices and is generally more complex. Depending on the composition of an erodible device (type of polymer, drug loading and additives) and geometry (size and shape), numerous mass transport phenomena and chemical reaction phenonema affect the resulting drug release kinetics such as:

- Water instruction into the device
- Drug dissolution
- Polymer degradation
- Creation of aqueous pores
- Diffusion of drug/polymer degradation
- Crystallization of polymer degradation productsand /or drug within the drug delivery device
- Micro-enviromental pH changes
- Osmotic effects
- Polymer swelling
- Convection processes
- Adsorption / desorption process

Physicochemical characteristics of biodegradable drug delivery systems

Physicochemical characterization techniques

Characterization of the physical and chemical changes involved in the drug release is essential in developing a mathematical model. A standard technique used for studying the polymer degradation is Gel Permeation Chromatography (GPC). It allows monitoring the polymer molecular mass changes during erosion and drug release. This study is particularly important because many theories are available which link the drug diffusion coefficient inside the degradable polymers directly to the polymer molecular mass as small chains offers less restriction for the drug diffusion than long chains (Spenlehauer G et al., 1988; Crotts et al., 1997). Another simple but effective technique to characterize the erosion of a biodegradable polymer matrix is the recording the mass loss during an erosion experiment (Gopferich A and Langer R., 1993a; Gopferich A., 1997).

Differential Scanning Calorimetry studies can throw light on the crystallinity and the glass transition temperature of the polymer. Wide angle X-ray diffraction (Gopferich A and Langer R., 1993b; Gopferich A et al., 1995) is often used to further characterize changes in crystallinity. Scanning electron microscopy can be useful to assess the microstructure of a polymer matrix during erosion which is important with respect to the interpretation of the experimental data. Also electron paramagnetic resonance spectroscopy and nuclear magnetic resonance imaging techniques have been used to characterize bioerodiable drug delivery systems (Maeder K et al., 1997a; Maeder K et al., 1997b). Photon Correlation Spectroscopy and other particle size measurements techniques can be applied to monitor the evolution of size of erodible devices versus time (Giunchedi P et al., 1998; Mueller RH et al., 1990).

Importance of type of polymer bond

The degradation velocity of the polymer depends on the type of the functional groups from which it is built. A brief list of some commonly used pharmaceutically relevant biodegradable polymers is shown in figure 2.16. The most reactive of all the type of bonds is the carboxylic acid anhydrides, followed by the ketal- and the ortho ester groups. Other degradable bonds hydrolyse by orders of magnitude slower than these. However, the type of monomers present (Park TG., 1995), co-polymerization (Dahlmann J et al., 1990),

neighbouring groups (Kirby AJ ., 1972), pH and the ions present in the degradation medium (Sykes P., 1975; Shih C., 1984) can significantly affect the hydrolysis rate.

Effect of pH on the polymer degradation

For many pharmaceutically relevant polymers, the pH of the micro- environment is a very important factor influencing the degradation kinetics. Hydrolysis rates can vary by orders of magnitude at different pH values (Kirby AJ., 1972; Leong KW et al., 1984). Poly (lactic acid) is a good example how pH can affect the polymer degradation. The hydrolysis of poly (lactic acid) can either be acid or base catalysed. The monomer generated by erosion, lactic acid, is a strong acid and is highly water soluble and this along with its low pK_a can lead to drastic changes in the micro environmental pH within the matrix pores during erosion. This is of great importance because the decreased pH can cause autocatalytic effects (Ottenbrite RA et al., 1992), which can lead to acceleration in the polymer degradation inside the PLA based drug delivery device compared to the surface (Li SM et al., 1990; Gopferich A., 1996). This phenomenon is particularly interesting in the case of small devices like microparticles. The diffusion pathways for hydroxide ions from the external release medium (e.g. phosphate buffer pH 7.4) into the center of the system is relatively short. Thus, the rate at which the hydroxide ions diffuse into the system might be significantly high to neutralize the hydrogen ions generated by polymer degradation resulting in suppression of autocatalysis.

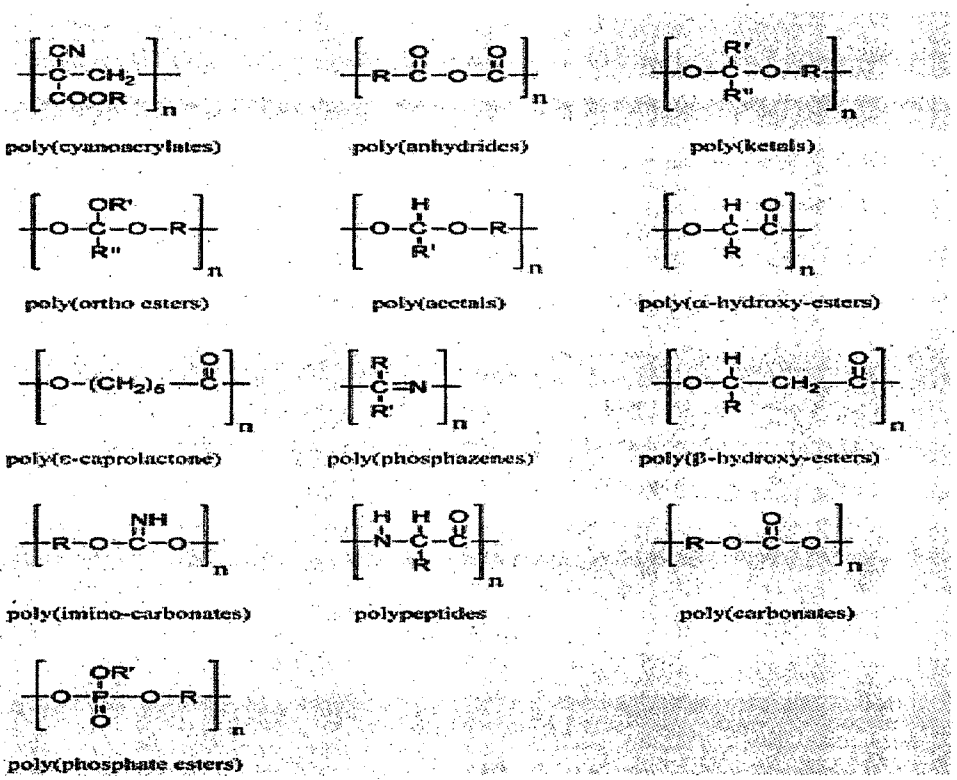


Figure 2.16: Functional groups of some commonly pharmaceutically relevant biodegradable polymers

Surface versus bulk erosion

Two different erosion mechanisms are proposed for the biodegradable polymers

1. Surface or heterogeneous erosion
2. Bulk or homogenous erosion

In case of surface eroding systems, polymer degradation is much faster than water intrusion into the polymer bulk. Degradation occurs mainly in the outer layers of the delivery device. Hence, erosion affects only the surface and not the inner parts of the matrix (heterogeneous process). Bulk eroding polymers in contrast degrade slowly and water uptake by the system is much faster than the polymer degradation. Hence the whole system is rapidly hydrated and the polymer chains are cleaved throughout the device. Hence, erosion is not restricted to the polymer surface (homogenous process). As a basic rule, polymers that are build from very reactive groups tend to degrade fast and are surface eroding, whereas polymers containing less reactive functional groups tend to be bulk

eroding. Polyanhydrides are examples of surface eroding polymers, while poly (lactide) (PLA) and poly (lactide-co - glycolide) (PLGA) are examples of bulk eroding systems.

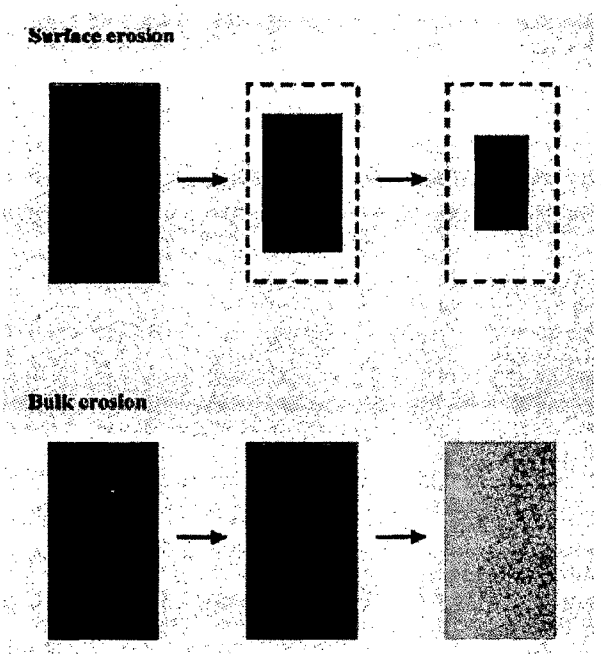


Figure 2.17: Schematic representation of the principle of surface erosion and bulk erosion

Drug	Drug Delivery system	Route of administration/ Function	Reference
Aciclovir	PLGA 50:50 microspheres	Intravitreal	Sanchez CM et al., 2004
Somatostatin acetate	PLGA 50:50 microspheres	Intramuscular depot	Herrmann J et al., 1998
Doxorubicin	PLGA 50:50 millirods PLGA – drug conjugates	Liver implant Parenteral	Qian F et al., 2003
Nerve growth factor	PLGA 50:50 microspheres PLGA 85:15 microspheres	Parenteral	Cao X., 1999
10- Hydroxycamptothecin	PLGA 50:50 microspheres	Parenteral	Shenderova A et al., 1997
Pentamidine	PLGA 50:50 microspheres in Poly vinyl alcohol hydrogel	Parenteral	Mandal T et al., 2002
Vinca alkaloids	PLGA 50:50 microspheres	Parenteral	Marinina J et al., 2000
Darbepoetin Alfa	PLGA 50:50 microspheres	Parenteral	Burke PA et al., 2004
Deslorelin	PLGA 50:50 microspheres	Pulmonary	Koushik K et al., 2004
Clodronate	PLGA 50:50 microspheres	Parenteral	Perugini P et al., 2001
Human Growth Hormone	PLGA 50:50 microspheres	Parenteral	Capan Y et al., 2003
Bisphosphonates	PLGA 50:50 Implant	Intramuscular Implant	Weidenauer U et al., 2004

Table 2.12: Use of Poly (lactide-co-glycolide) as drug delivery systems

2.5 NANOSUSPENSIONS AS DRUG DELIVERY SYSTEMS

Introduction

Nanosuspensions of drugs are sub-micron colloidal dispersions of pure particles of drug, which are stabilized by surfactants (Na GC., 1999). The use of nanosuspensions in parenteral drug delivery is a fairly new concept. For many decades, coarse solid suspensions (10–100μm) have been produced for intramuscular or subcutaneous delivery of poorly water-soluble drugs. Examples include penicillin G benzathine (BICILLIN L-A by Wyeth-Ayerst), prepared by the reaction of dibenzylethylene diamine with two molecules of penicillin G, dexamethasone acetate (DECADRON-LA, by Merck), and methylprednisolone acetate (DEPOMEDROL, Pfizer), which are administered intramuscularly. Insulin has long been formulated with zinc as a suspension for subcutaneous delivery (for example, HUMULIN, ILETIN, LENTE and NOVOLIN, developed and manufactured by Lilly).

Physicochemical characteristic	Potential benefits
Increased drug amount in dosage form without harsh vehicles (extreme pH, co-solvents)	Intravenous: reduced toxicity, increased efficacy
Reduced particle size: increased drug dissolution rate	Oral: increased rate and extent of absorption, increased bioavailability of drug. area under plasma versus time curve, onset time, peak drug level, reduced variability, reduced fed/fasted effects. Pulmonary: increased delivery to deep lung
Solid state: increased drug loading	Reduced administration volumes; essential for intramuscular, subcutaneous, ophthalmic use
Solid state: increased stability	Increased resistance to hydrolysis and oxidation, increased physical stability to settling
Particulate dosage form	Intravenous: potential for intravenous sustained release via monocyte phagocytic system targeting, reduced toxicity, increased efficacy. Oral: potential for reduced first-pass hepatic metabolism

Table 2.13: Benefits of nanosuspensions

Methods of Preparing drug nanosuspensions

Homogenization

Homogenization involves the forcing of a suspension under pressure through a valve (Pandolfe WD et al., 1982; Schultz, S et al., 2002) that has a narrow aperture (Muller RH and Peters K., 1998; Liedtke S et al., 2000). As per Bernoulli's law, the high velocity of the suspension that results from flow past the constriction is compensated by a reduction in static pressure. This, in turn, causes bubbles of water vapor to form, which then collapse as they exit the valve. These cause cavitation-induced shock waves, which crack the particles (Figure 2.18) Turbulent flow and shear, have also been investigated as mechanisms for reducing particle size through homogenization (Jahnke S et al., 1998; Mohr KH., 1987).Crystals are susceptible to such breakage to varying degrees. Pure crystals have a theoretical tensile strength that is 100–1,000 times stronger than what is observed in practice. However, the inevitable crystal defects — dislocations and impurities — weaken mechanical behavior by migrating and accumulating at crystal-grain boundaries, which furnishes a nidus of weakness (Grant D and York P., 1986; Duddu SP and Grant D., 1995).

Homogenization is advantageously utilized to exploit three consequences of rapid precipitation to either further reduce particle size or to resolve other potential difficulties (Kipp JE., 2003). First, the crystal defects induced by rapid precipitation render the crystal more susceptible to rupture by the subsequent mechanical shock of homogenization. Second, fracture mechanics predict that the dendritic morphology itself is more susceptible to breakage because of the narrow dimension induced, which must bear the full applied force. Third, the mechanical energy supplied by the homogenizer enables initially formed, unstable amorphous particles that result from rapid precipitation to undergo subsequent crystallization to a stable state.

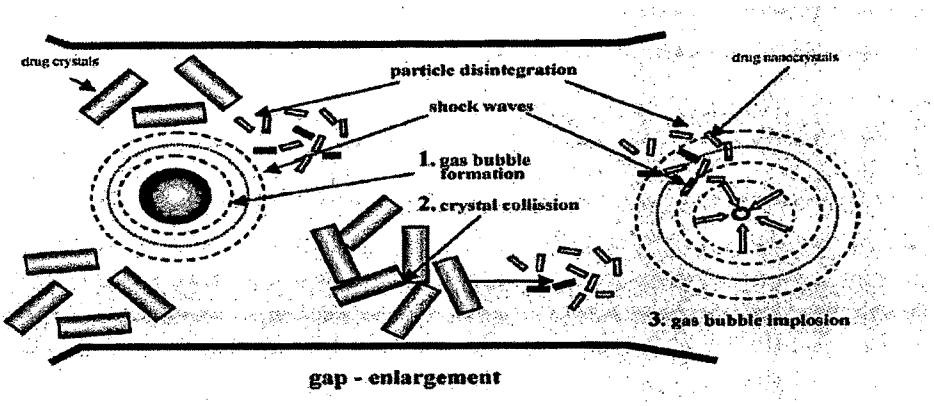


Figure 2.18: Principle diminution mechanism in homogenisation gap of a piston-gap homogeniser of the DissoCubes® technology.

Wet milling

Another manufacturing technique for preparing nanosuspensions is wet milling, in which the active agent, in the presence of surface stabilizer(s), is comminuted by milling media. Particle size here is determined by stress intensity and the number of contact points. The stress intensity is a function of the kinetic energy of the grinding beads, and the number of contact points can be increased by utilizing smaller grinding media. A drive shaft, attached to rotating disks, provides the energy to a charge of crosslinked polystyrene beads to comminute the drug crystals by a compression–shear action (Liversidge ME et al., 2003).

Conventional milling and precipitation processes generally result in particles much greater than 1μm. Techniques were later refined by Liversidge and coworkers at NanoSystems (now part of Elan Corporation), to enable milling of solid drug particles (NANOCRYSTAL technology) to well below 1μm (Liversidge GG et al., 1992). NanoSystems approach relies on the use of the ball mill or pearl mill, in which milling beads of sizes ranging from 0.4 to 3mm are used. These beads may be composed of glass, zirconium salts, ceramics, or plastics (e.g., cross-linked polystyrene). Even with very hard grinding materials, however, erosion of the milling material may be an issue (Redzisqewski, P., 1997; Joost B and Schwedes J., 1996). After separation of the grinding medium from the suspension, fine particles may potentially remain. The time required for milling depends on the hardness and brittleness of the drug material in

comparison to the milling particle size and form. In the pharmaceutical field, the term "nanoparticle" has been rather loosely applied to structures less than 1μm in diameter. They can be produced by either chemical or mechanical means, and characterized by conventional analytical methods such as microscopy or light scattering.

Other techniques.

Yet another technique involves the spraying of a drug solution in a volatile organic solvent into a heated aqueous solution. Rapid solvent evaporation produces drug precipitation in the presence of surfactants (Sarkari M., 2002). Lyophilization of a nanosuspension can be performed to overcome either physical or chemical incompatibility, permitting recovery of the original particle size after reconstitution (Peters K., 2000). Sterilization can be performed by aseptic processing of previously sterilized components (Toguchi H et al., 1999), membrane filtration for particles sufficiently small (Zheng, JY and Bosch, JW., 1997; Konan, YN et al., 2002), or, for drugs that will withstand it, steam sterilization (Na GC et al.,1999) or γ-irradiation. The manufacturing process is built around the unit operations of particle-size reduction and sterilization. The process can be varied to permit the use of previously sterilized raw material, sterile addition of excipients before filling, and lyophilization. The unit operations of sterile crystallization, aseptic dispersion of previously sterilized drug and aseptic milling are all used in currently manufactured microsuspensions, such as testosterone, insulin, dexamethasone acetate and penicillin G (Floyd AG and Jain S et al., 1996).

Characterization techniques

Tests for the quality of nanosuspensions are selected on the basis of the performance required. For pharmaceutical use, a suspension must have a minimal tendency to agglomerate, which could lead to the formation of a hard cake. A suspension with slow sedimentation rate is acceptable, perhaps even preferable, provided the product is re-suspendable and homogeneous. In addition, the product must be non-toxic and non-irritating. A suspension used for injectables must also be sterile, non-pyrogenic, syringeable, injectable, isotonic and non-haemolytic (Akers MJ et al., 1987). A variety of techniques are used to measure particle-size distribution, because of their different operating principles and features. For example, laser light diffraction can be used because it is fast and suitable for screening large numbers of samples, acquiring data in the useful range of 0.02–2,000μm (Barber TA., 1993). However, because it is an ensemble (Weiner BB., 1996) rather than a single particle- counting technique, it should be

calibrated against a more accurate method. When the particles are smaller than the wavelength of visible light, optical microscopy cannot be used as a reference method. But in this range, field emission low-voltage scanning electron microscopy can be used to image individual particles. However, analysing a sufficiently large statistical number of particles to achieve reasonable levels of precision becomes tedious. Photon-correlation spectroscopy is rapid, but only covers the range of 0.02–3μm (Lines RW., 1996). Light-obscuration counters have a lower sensitivity cut-off of 0.5μm. In addition to characterizing the mean particle size and particle-size distribution, focus should also be directed to characterizing the very high end particle size, especially for injectables. Although not intended for this purpose, the United States Pharmacopeia microscopic particle matter test, with limits on number of particles greater than 10μm and 25μm, is serviceable (<788> USP 27).

Finally, visualization of particle shape by atomic-force microscopy has been reported (Shi HG., 2003). In the course of the development of a nanosuspension formulation, one first screens various surfactant packages to achieve the desired particle size, with a narrow distribution in size. Once achieved, accelerated stability tests are performed, which challenges the system both thermally and mechanically. The rapid assessment of sedimentation potential by near-infrared (Kuentz M and Rothlisberger D., 2003) is possible. Polymorph stability to processing is assessed by differential scanning calorimetry (Giron D., 1995; Yoshii K., 1997) and X-ray diffraction. On-line monitoring of particle size by near-infrared (Higgins JP., 2003) is a possibility. The bioavailability of a nanosuspension, by any route of administration, ultimately depends on the dissolution of the drug. *In vitro* dissolution testing in a bio-relevant medium (Nicolaides E., 2001; Dressman, JB and Reppas C., 2000) furnishes guidance as to the potential pharmacokinetics to be expected in vivo, as explained in the applications section. Dissolution rates can be affected by pH and the nature of the polymorph, which can therefore affect pharmacokinetics.

Applications

Nanosuspensions are used to advantage in diverse dosage forms. In some cases, their small size and increased surface area leads to an increased dissolution rate and increased bioavailability. In other cases, their particulate nature dictates targeting of the Monocyte Phagocytic System (MPS), with unusual pharmacokinetic consequences. Nanosuspension drugs that are marketed or currently in clinical trials are listed in Table 2.14.

Oral: In general, oral suspensions are selected because of the superior taste-masking of the particulate form, or difficulty experienced by the very young and old in swallowing tablets (Donovan M and Flanagan D., 1996), or to overcome solubility problems. Oral nanosuspensions have been specifically used to increase the rate and extent of the absorption of drugs (Jia L et al., 2002) due to their solubility limitations. In comparison with a conventional 10μm danazol suspension, a 169-nm nanoparticulate dispersion showed higher C_{max} (3.01 versus 0.20μg per ml) and area under the curve (AUC) (16.5 versus 1.0μg•h per ml) in a pharmacokinetic study in dogs. The bioavailability of the nanosuspension was equivalent to that of a cyclodextrin solution formulation, indicating that the dissolution-rate-limited bioavailability observed with the 10μm suspension had been overcome. The absolute bioavailability was 82.3 ± 10.1% of an intravenous control injection. By reducing the size of particles to the sub-micron level, the uptake of intact gastrointestinal polymeric particles has been shown to occur preclinically, by mechanisms involving M-cells in Peyer's patches of the gastrointestinal lymphoid tissue (Clark M et al., 2001). This uptake pathway communicates with the mesenteric lymph ducts, and empties via the thoracic duct into the systemic blood circulation. This approach therefore provides a route for avoiding first-pass metabolism, as well as for targeting sanctuaries of lymphatic-mediated diseases. The low drug uptake by this pathway might be enhanced, as shown in cell-based studies, by coating drug particles with agents, such as vitamin B12 (Russell JGJ et al., 1999) , that dock into transporter receptors on the intestinal epithelium. The use of surfactants has also been found to have an impact on decoupling the intestinal P-glycoprotein drug-efflux pump as well as interfering with lipidic chylomicron transport systems.

Injectable: Injection of poorly water-soluble drugs is often approached by formulating drugs with excessive amounts of co-solvents, which provoke anaphylactoid reactions (Bittner B and Mountfield RJ., 2002), pain on injection (Theis JG., 1995) and precipitation of drug following dilution of the vehicle in the blood. Lipids might work for drugs that are lipid-soluble, which often is not the case for drugs having high crystal energies. To establish a more comprehensive approach, therefore, the formulation of injectable drugs as nanosuspensions has emerged. The approach is generally applicable, provided that the aqueous solubility is below several hundred μg per ml, to prevent Ostwald's ripening. Successful formulation has been reported as applied to antineoplastic agents, anaesthetic agents, antifungals and antibacterials, as well as for agents for malignant hyperthermia and cancer pain. Several pharmacokinetic profiles can result

following the injection of nanosuspensions. If the particles dissolve in the blood readily, both the pharmacokinetics, and therefore tissue distribution, will be equivalent to those for the solution formulations (Clement MA., 1992), affording a relatively fast onset of action. Alternatively, depot delivery via subcutaneous, intramuscular or intradermal routes offers prolonged drug release, because of the ability to load more drug safely into a small injectable volume. The greater loading capacity (up to 30%) distinguishes nanosuspensions from polymeric nanoparticulate vehicles. The small size of the particles results in significantly faster dissolution than microsuspensions, provided that post-injection aggregation does not occur. Because drug dissolution is often the rate-limiting step in systemic drug uptake from a depot (Zuidema J et al., 1988), nanosuspensions often result in higher peak plasma levels as well. But because dissolution is not instantaneous, as it would be for a solution dosage form, there is less toxicity associated with nanosuspensions, enabling high loading with safety.

The pharmacokinetic parameters of a nanocrystal suspension of itraconazole, was compared with those of the commercial Sporanox solution. The nanocrystal suspension occupied a larger volume of distribution (1,677 ± 827 l versus 796 ± 185L) and was cleared more slowly (3.35 ± 1.8 l per h versus 22.9 ± 5.7 l per h) to give a longer half-life (346 ± 225 h terminal versus 35.4 ± 29.4 h mean and 30 h terminal) and larger area under the plasma concentration curve for the first 24 hours, AUC24, (51,558 ± 10,635μg*h per L versus 30,605 ± 8,961μg*h per L). This behavior is consistent with MPS depot behavior, resulting in prolonged delivery for the nanosuspension. The nanocrystal suspension was well tolerated.

Pulmonary To target the deep lung, respirable aerosols should have mean aerodynamic diameters of 1–5μm (Chan HK and Gonda I., 1988). In conventional solid-in-liquid dispersions, the solid drug has a particle size that is comparable to that of the aerosol. There is therefore statistical inhomogeneity in the partitioning of drug particles among the carrier droplets (Wiedmann TS et al., 1997). Nanosuspensions would ameliorate this by increasing the number of particles per droplet (Jacobs C and Muller RH., 2002). In fact, aerosol cascade impactor studies have shown significantly higher respirable fractions and lower unwanted systemic uptake via throat deposition for nanosuspensions compared with micronized formulations. Respirable dose was increased from 227μg to 421μg by decreasing particle size from 4.4μm to 0.73μm, prepared by homogenization. The use of already approved surfactants can be used for drug nanosuspensions (Dailey LA., 2003), to avoid safety problems posed by

polymeric nanoparticulates. In a Phase I clinical trial, nebulized nanocrystal (75–300 nm) budesonide suspension had double the C_{max}, and almost half the T_{max}, of larger, 4,400- nm sized Pulmicort Respules. The total amount absorbed, as indicated by AUC, was comparable. The faster absorption, which resulted in higher peak plasma levels, could have been attributable either to more rapid dissolution or faster entry to the blood via access to the additional vasculature of the peripheral lung.

Central nervous system: Nanosuspensions afford a means of administering increased concentrations of poorly water-soluble drugs to the brain with decreased systemic effects (Dailey LA., 2003). Significant efficacy has been shown with microparticulate busulfan in mice administered intrathecally (Grossman SA and Krabak MJ., 1999). The work has advanced to Phase I in patients afflicted with neoplastic meningitis, administered via an Ommaya Reservoir for intraventricular delivery, and via lumbar puncture. The drug was well tolerated and resulted in delayed progression of disease (H. Friedman, personal communication). Epidural injection of a 10% butamben suspension for cancer pain was well tolerated in dogs and humans). Future work will probably also involve less invasive routes, utilizing either passive targeting (via Pegylation, as has been done for liposomes) or active targeting to the brain (Shulman M., 1990; Kreuter J., 2001) following intravenous administration of nanosuspensions. In these latter publications, it was found that use of the agent Polysorbate 80 in the formulation led to deposition of apolipoprotein E on the nanoparticles, which facilitated brain uptake by receptors on the brain endothelial cells.

Drug	Indication	Drug delivery company	Pharma company	Route	Status
Paclitaxel	Anticancer	American BioScience	American Pharmaceutical Partners	Intravenous	Phase III
Rapamune	Immuno- suppressant	Elan Nanosystems	Wyeth	Oral	Marketed
Emend	Anti-emetic	Elan Nanosystems	Merck	Oral	Marketed
Cytokine inhibitor	Crohn's disease	Elan Nanosystems	Cytokine Pharma Sciences	Oral	Phase II
Diagnostic Agent	Imaging agent	Elan Nanosystems	Photogen	Intravenous	Phase I/II
Thymectacin	Anticancer	Elan Nanosystems	NewBiotics./Ilex Oncology	Intravenous	Phase I/II
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	Phase I
Busulfan	Anticancer	SkyePharma	Supergen	Intrathecal	Phase I
Budesonide	Asthma	Elan Nanosystems	Sheffield Pharmaceuticals	Pulmonary	Phase I
Silver	Eczema, atopic dermatitis	NUCRYST	Self-developed	Topical	Phase I
Calcium phosphate	Mucosal vaccine adjuvant for herpes	BioSante	Self-developed	Oral	Phase I
Insulin	Diabetes	BioSante	Self-developed	Oral	Phase I

Table 2.14: Solid-particulate-nanosuspension-based formulations in development and in the market

2.6 SOLID LIPID NANOPARTICLES AS DRUG DELIVERY SYSTEMS

Introduction

In the middle of the 1990s, the attention of different research groups has focused on alternative nanoparticles made from solid lipids, the so-called solid lipid nanoparticles (SLN or lipospheres or nanospheres) (Siekmann B and Westesen K., 1992; Gasco MR., 19903; Schwarz C et al., 1994; Cavalli R et al., 1996; Muller RH et al., 1995). The SLN combine the advantages of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability) while at the same time minimizing the associated problems. SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonary, and rectal) have been developed and thoroughly characterized in vitro and in vivo (Pinto JF et al., 1999; Dingler A et al., 1999; Demirel M et al., 2001; Wissing SA et al., 2002; Morel S et al., 1994). A first product has recently been introduced to the Polish market (Nanobase, Yamanouchi) as a topically applied moisturiser. At the turn of the millenium, modifications of SLN, the so-called nanostructured lipid carriers (NLC) and the lipid drug conjugate (LDC) nanoparticles have been introduced to the literature (Muller RH et al., 2002; Radtke M et al., 2000). These carrier systems overcome observed limitations of conventional SLN.

SLN are particles made from solid lipids (i.e. lipids solid at room temperature and also at body temperature) and stabilized by surfactant(s). By definition, the lipids can be highly purified triglycerides, complex glyceride mixtures or even waxes. The main features of SLN with regard to parenteral application are the excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release (fast or sustained) depending on the incorporation model, good tolerability and site specific targeting. Potential disadvantages such as insufficient loading capacity, drug expulsion after polymorphic transition during storage and relatively high water content of the dispersions (70–99.9%) have been observed. The drug loading capacity of conventional SLN is limited (generally up to approximately 25% with regard to the lipid matrix, up to 50% for special actives such as Ubidecarenone) by the solubility of drug in the lipid melt, the structure of the lipid matrix and the polymorphic state of the lipid matrix (Westesen K et al., 1993; Westesen K et al., 1997; Westesen K et al., 2000; Siekmann B et al., 1994). If the lipid matrix consists of especially similar molecules (i.e. tristearin or tripalmitin), a perfect crystal with few imperfections is formed. Since incorporated drugs are located between fatty acid chains, between the lipid layers and also in crystal

imperfections, a highly ordered crystal lattice cannot accommodate large amounts of drug. Therefore, the use of more complex lipids (mono-, di-, triglycerides, different chain lengths) is more sensible for higher drug loading.

The transition to highly ordered lipid particles is also the reason for drug expulsion. Directly after production, lipids crystallize—partially—in higher energy modifications (α, β) with more imperfections in the crystal lattice (Freitas C et al., 1999; Hagemann JW., 2004). The preservation of the a-modification during storage and transformation after administration (e.g. by temperature changes) could lead to a triggered and controlled release and has recently been investigated for topical formulations (Jenning V et al., 2000). However, if a polymorphic transition takes place during storage, the drug will be expelled from the lipid matrix and it can then neither be protected from degradation nor released in a controlled way.

Production of lipid nanoparticles

Different approaches exist for the production of finely dispersed lipid nanoparticle dispersions. In this section, the various methods are described briefly, also with regard to scaling up possibility, a prerequisite for the introduction of a product to the market.

High pressure homogenisation (HPH)

HPH is a suitable method for the preparation of SLN, and can be performed at elevated temperature (hot HPH technique) or at or below room temperature (cold HPH technique) (Cortesi R et al., 2002; Lim SJ et al., 2002; Siekmann B et al., 2002). The particle size is decreased by cavitation and turbulences. Briefly, for the hot HPH, the lipid and drug are melted (approximately 5°C above the melting point of the lipid) and combined with an aqueous surfactant solution having the same temperature. A hot preemulsion is formed by high speed stirring. The hot pre-emulsion is then processed in a temperature controlled high pressure homogenizer, generally a maximum of three cycles at 500 bar are sufficient. The obtained nanoemulsion recrystallises upon cooling down to room temperature forming SLN, NLC or LDC. The cold HPH is a suitable technique for processing temperature labile drugs or hydrophilic drugs. Here, lipid and drug are melted together and then rapidly ground under liquid nitrogen forming solid lipid microparticles. A pre-suspension is formed by high speed stirring of the particles in a cold aqueous surfactant solution. This pre-suspension is then homogenized at or below room temperature forming SLN, NLC or LDC, the homogenizing

conditions are generally five cycles at 500 bar. The influence of homogenizer type, applied pressure, homogenization cycles and temperature on particle size distribution has been studied extensively (Muller RH et al., 1993; Liedtke S et al., 2000) Both HPH techniques are suitable for processing lipid concentrations of up to 40% and generally yield very narrow particle size distributions (Polydispersity index < 0.2) (Lippacher A et al., 2001).

Production of SLN via microemulsions

GAsco and coworkers have developed and optimised a suitable method for the preparation of SLN via microemulsions which has been adapted and/or modified by different labs (Cavalli R et al., 1998; Igartua M et al., 2002). Firstly, a warm microemulsion is prepared by stirring, containing typically c10% molten solid lipid, 15% surfactant and up to 10% cosurfactant. This warm microemulsion is then dispersed under stirring in excess cold water (typical ratio 1:50) using an especially developed thermostated syringe. The excess water is removed either by ultra-filtration or by lyophilization in order to increase the particle concentration.

Preparation by solvent emulsification-evaporation or -diffusion

Different academic groups have attempted the production of SLN via precipitation. In the solvent emulsification-evaporation (Dubes A et al., 2003), the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under reduced pressure. Upon evaporation of the solvent, the lipid precipitates forming SLN. An important advantage of this method is the avoidance of heat during the preparation, which makes it suitable for the incorporation of highly thermolabile drugs. Problems might arise due to solvent residues in the final dispersion; Sjostrom et al. have calculated the amount of toluene residues as 20–100 ppm in final dispersions. Also, these dispersions are generally quite dilute, because of the limited solubility of lipid in the organic material. Typically, lipid concentrations in the final SLN dispersion range around 0.1 g/l, therefore, the particle concentration has to be increased by means of, e.g. ultra-filtration or evaporation. In the solvent-diffusion technique, partially watermiscible solvents (e.g. benzyl alcohol, ethyl formate) are used (Trotta M et al., 2003; Hu FQ et al., 2002). Initially, they are mutually saturated with water to ensure initial thermodynamic equilibrium of both liquids. Then, the lipid is dissolved in the water-saturated solvent and subsequently emulsified with solvent-saturated aqueous surfactant solution at elevated temperatures. The SLN precipitate after the addition of excess water (typical ratio: 1:5–1:10)

due to diffusion of the organic solvent from the emulsion droplets to the continuous phase. Similar to the production of SLN via microemulsions, the dispersion is fairly dilute and needs to be concentrated by means of ultra-filtration or lyophilization. Average particle sizes around 100 nm and very narrow particle size distributions can be achieved by both solvent evaporation methods.

Preparation by w/o/w double emulsion method

Recently, a novel method based on solvent emulsification–evaporation for the preparation of SLN loaded with hydrophilic drugs has been introduced to the scientific community (Cortesi R et al., 2002). Here, the hydrophilic drug is encapsulated—along with a stabiliser to prevent drug partitioning to the external water phase during solvent evaporation—in the internal water phase of a w/o/w double emulsion. This technique has been used for the preparation of sodium cromoglycate- containing SLN, however, the average size was in the micrometer range so that the term "lipospheres" in the sense as a term for nanoparticles is not used correctly for these particles.

Preparation by high speed stirring and/or ultra sonication

The SLN were developed from lipid microparticles produced by spray congealing followed by lipid nanopellets produced by high speed stirring or sonication (Eldem T et al., 1991). A great advantage of this method is the fact that the equipment is common in every lab and the production can easily be done. The problem of high speed stirring was a broader particle size distribution ranging into the micrometer range. This could lead to physical instabilities such as particle growth upon storage. This could be improved by higher surfactant concentrations, which in order might be correlated with toxicological problems after parenteral administration. A further disadvantage is potential metal contamination due to ultra sonication.

Stability of SLN dispersions

The physical stability of SLN dispersions has been investigated intensively, e.g. by measurements of particle size (photon correlation spectroscopy, PCS; laser diffraction, LD), charge (ZP) and thermal analysis (differential scanning calorimetry, DSC). The average diameter of the main population remained between 160 and 220 nm for the investigated period. Freitas and Muller investigated the effect of light and temperature on the physical stability of SLN dispersions composed of 10% tribehenate and 1.2% poloxamer 188

(Freitas C and Muller RH., 1998). They found that particle growth could be induced by an input of kinetic energy (light, temperature) to the system. Storage under artificial light lead to gelation of the system within 7 days of storage, under day light within 3 months and in darkness particle growth started after 4 months storage. The gelation was accompanied by a decrease in ZP from -24.7 to below -18 mV. The influence of the storage temperature on particle size has also been analyzed. The authors found that the particle size measured by LD increased rapidly at elevated temperatures and remained stable for more than 180 days when refrigerated. Again, particle growth could be correlated to a decrease in ZP from -24.7 to approximately -15 mV. Freitas and Muller have also correlated the physical stability of the aforementioned SLN formulation with the polymorphic state of the lipid (Freitas C and Muller RH., 1999). After hot HPH, the lipid recrystalizes as a complex mixture of β', α, sub α polymorphs. The input of kinetic energy causes a transformation to β accompanied by gel formation. By inhibition of this transformation (refrigerated, dark storage), this transformation could be avoided. These studies show that the development of optimal storage conditions can improve the physical stability of previously regarded unstable SLN formulations tremendously.

Apart from optimized storage conditions of labile SLN dispersions, they can also be spray-dried or lyophilized. For spray-drying, a melting point of the lipid matrix of >70°C is a pre-requisite. Typically, protectors such as trehalose are added to the dispersion in concentrations of about 20–25%. For best reconstitution effects, SLN concentration in the spraying medium should be approximately 1%. The influence of lipid type and concentration, carbohydrate type and concentration, redispersion medium and spraying medium have been investigated by Freitas and coworkers (Freitas C and Muller RH., 1998). Lyophilization can be employed as an alternative very sensitive drying method. The process has been optimized with regard to operating conditions, lipid concentration, type and concentration of cryoprotectant and redispersing conditions (Zimmermann E et al., 2000). Heiati and coworkers (Heiati H et al., 1998) have investigated the effect of cryoprotective sugars on the size of neutral and negatively charged SLN after lyophilization and reconstitution. The azidothymidine palmitate (AZT-P) loaded SLN were composed of trilaurin, stabilised with lecithin and they were prepared by solvent emulsification–evaporation and subsequent HPH. They found that trehalose was the most effective cryoprotectant in a sugar/lipid ratio of 3:9 for neutral SLN and 2:6 for negatively charged SLN. Also, trehalose was most effective for preventing drug expulsion upon reconstitution. Lim and coworkers showed for all trans retinoic acid-loaded

SLN excellent redispersion characteristics. Here, the PCS diameter increased merely from 182 to 265 nm and the Polydispersity index from 0.173 to 0.200 upon redispersing. No changes in ZP and in drug loading were observed. For SLN based on calixarenes, difficulties in redispersing after lyophilization have been observed, i.e. these particles require up to 1 h of ultrasonic treatment, however no cryoprotectant was added to the formulations, so that these results have to be regarded as preliminary.

Incorporation of drugs

The drug can be incorporated between fatty acid chains, between lipid layers or in imperfections. Depending on the drug/lipid ratio and solubility, the drug is located mainly in the core of the particles, in the shell or molecularly dispersed throughout the matrix. By optimizing of the formulation, long term physical stability can be achieved. The solid lipid core of SLN should increase the chemical stability of incorporated drugs and protect them from degradation. Therefore, entrapment efficiency and long term retention of the drug in the lipid matrix have to be ensured.

Characterization of solid lipid nanoparticles

An adequate characterization of the solid lipid nanoparticles is necessary for the quality control of the quality of the product. The major parameters that can affect the quality of the final SLN dispersion are shown in figure 2.19.

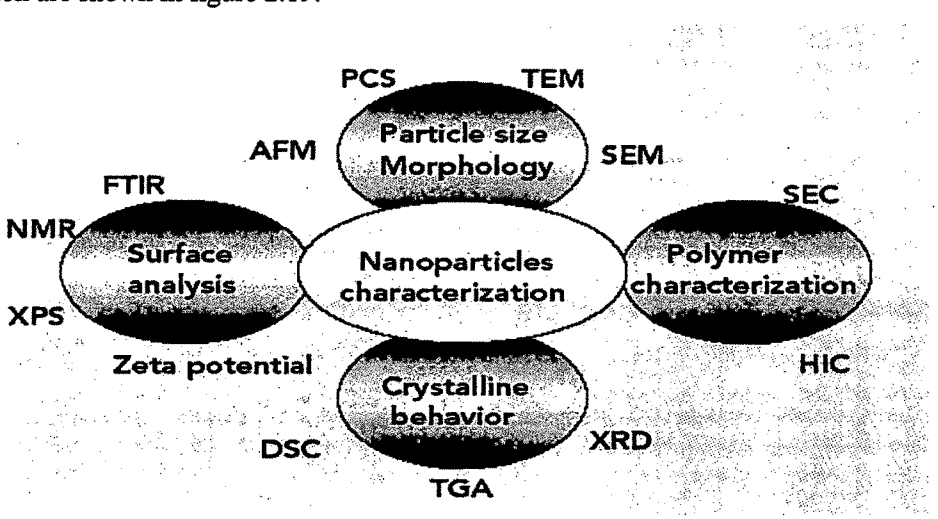


Figure 2.19: Various characterization techniques available for solid lipid nanoparticles

Parameter	Technique used	Remarks
Particle size	*Photon correlation spectroscopy*	Commonly used (sensitivity few nanometers to 3μm)
	Laser diffraction	Commonly used (sensitivity nanometer range to lower millimeter range)
	Coulter Counter	Difficult to assess small nanoparticles and requires electrolytes which can destabilize the colloidal dispersions
	Atomic force microscopy	Mostly used for biological samples (sensitivity upto 0.01nm). Requires immobilization of the nanoparticles before imaging
Determination of crystallinity	*Differential Scanning Calorimetry*	Based on the fact that different lipid modifications possess different melting points and melting enthalpies. Measurement in dispersion state recommended.
	X-ray scattering	Possible to measure the length of the long and short spacings of the lipid lattice. Sensitivity and long measurement times problems can be overcome by synchrotron irradition

Parameter	Technique used	Remarks
Determination of crystallinity	*Infrared spectroscopy (IR)*	-
	Raman spectroscopy (RS)	-
Rheometry	*Cone and plate type rheometers*	Characterization of viscoelastic properties
	Nuclear magnetic resonance (NMR)	Assigning the particular NMR signals to the particular molecules or their segments.
Determination of co existence of other colloidal structures	*Electron spin resonance (ESR)*	Requires addition of paramagnetic spin probes. Gives information about the micro viscosity and micro polarity.

Table 2.15: Characterization of SLN dispersions

Possible problems in SLN preparation and performance

Inspite of several advantages compared to other colloidal systems (easy scaling up, avoidance of organic solvents and high content of nanoparticles) there are some limitations of this carrier system also.

High pressure – induced drug degradation

High pressure homogenization (HPH) technique has been shown to reduce the molecular weight of polymers. High shear stress is the main reason that can be attributed to this detrimental action. High molecular weight compounds and long chain molecules are more sensitive than low molecular weight drugs and molecules with a spherical shape. For example HPH causes degradation of DNA and albumin.

Formation of supercooled melts

Formation of supercooled melts is not an unusual phenomenon in SLN systems. This phenomenon may take place when the sample is stored at a temperature below the melting

point of the lipid. Supercooled melts are not lipid nanosuspensions but emulsions. The main reason for the formation of supercooled melts is the size dependence of crystallization processes. Crystallization requires a critical number of crystallization nuclei to start. This critical number of molecules is less likely to be formed in small droplets and hence, the tendency of the formation of supercooled melts increases with the decrease in droplet size. The range of supercooling (the temperature difference between the melting and crystallization points) can reach 30-40°C in lipid dispersions. For example, the melting point of trilaurin is > 40°C, but in phospholipids / tyloxapol stabilized nanodispersions the lipid recrystallizes at temperatures below the freezing point of water (Westesen K and Bunjes H., 1995). In addition to the size of the nanoparticle, crystallization can be affected by emulsifiers, incorporated drug and other factors. Presence of supercooled melts can be detected by NMR studies.

Lipid modifications

It is sufficient to describe the physical state of the lipid as crystallized or non-crystallised, because the crystallized lipid may exist in several modifications of the crystal lattice. The lipid molecules have a higher mobility in the thermodynamically unstable configurations. Therefore, these configurations have lower density and ultimately, a higher capability to accommodate guest drug molecules. The advantage of higher incorporation rates in unstable modifications is paid off by an increased mobility of the drug. During storage, rearrangement of the crystal lattice may occur in favor of the thermodynamically stable configurations and this is often connected with the expulsion of the drug molecules which were initially incorporated inside the lipid lattice. The performance of the SLN system is mainly determined by the lipid modification, because this parameter triggers drug incorporation and drug release. Therefore, the utilization of the higher drug loading capacity in unstable configurations requires the development of strategies to prevent modifications during storage.

The crystallinity of Compritol SLN is comparatively high (70-80%) on the day of production. The liquid parts crystallize during storage. Stable SLN systems do not recrystallize during the storage period of 3 years and contain several lipid modifications (unstable α, sub α and the more stable β'). Semisolid samples contain only β' and α modification, while complete gelation lead to the complete transformation to the β' modification. The presence of liquid phases promotes the crystallization in the stable form because unstable crystals may dissolve and crystallize in the stable modification (Yoshino H et al., 1983). In most cases triglycerides

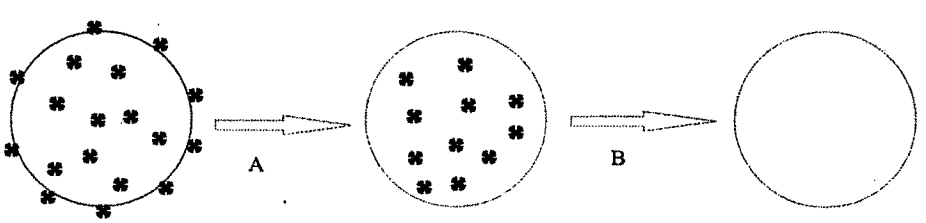
will crystallize in the α modification. The α → β' transformation can be retarded by surfactants like poloxamer (Garti N., 1988). Reports are available that a nitrogen atmosphere had similar effects and attributed this effect to the inhibition of the lipid hydrolysis (pH effect)

Gelling phenomenon

Gelling phenomena describe the transformation of low viscosity LSN dispersion into viscous gel. This process may occur very rapidly and unpredictably. In most cases, the gelling phenomenon is a irreversible process which involves the loss of the colloidal particle size. It can be stimulated by the intense contact of the SLN dispersion with other surfaces and shear forces. Some authors believe that the gel formation is connected wit the crystallization processes. Strange surfaces induce crystallization or change of modification of the lipid crystals. This process is connected with an increase of the particle surface due to the preferred formation of platelets (in the β modification). The surfactant molecules are no longer able to provide sufficient coverage of the new surfaces and therefore, particle aggregation is observed. Gelation can be retarded or prevented by the addition of co-emulsifying surfactants with high mobility.

Release of incorporated drugs

Next to the characterization of the carrier system SLN, the release characteristics have been studied by various research groups. It could be shown that the release profile can be influenced by modifications in the lipid matrix, surfactant concentration and production parameters. Mehnert and coworkers have performed intensive in vitro release studies and developed structural models for different release characteristics (Bensouda Y et al., 1989).



A: Rapid desorption of drug from the nanoparticle surface (burst release)
B: Slow controlled release of drug either by degradation of lipid matrix or diffusion of drug

Figure 2.20: Release mechanisms from nanoparticles

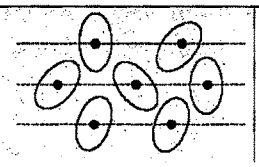
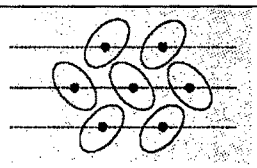
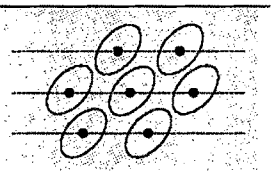
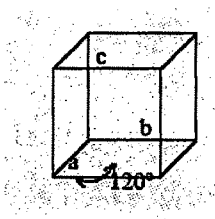
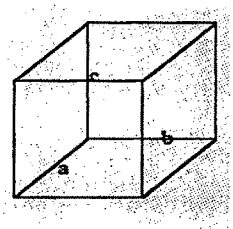
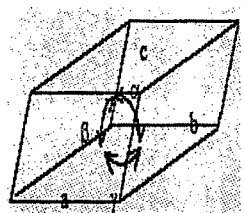
Characterization technique	Alpha	Beta-Prime	Beta
			
			
	Hexagonal (P)	Orthorhombic (P)	Triclinic (P)
X – ray Analysis	Tuning fork Acyl groups oriented at 90° to the plane the glyceryl group	Tuning fork Acyl groups are tilted of 68-70° from plane of the glyceryl group	Chair form Acyl groups are tilted about 59° from the plane of the glyceryl groups
Microscopy	Vertical chain orientation Platelet	Tilted chain orientation Fine needle	Tilted chain orientation Long needle
Infrared Spectroscopy	A singlet at 720 cm^{-1}	A doublet at A singlet at 717 cm^{-1}	719 and 727 cm^{-1}
Thermal Analysis	Thermodynamically most unstable Lowest melting point	Thermodynamically unstable Intermediate melting point	Most stable form Highest melting point

Table 2.16: Comparison of physical properties of the different lipid modifications

In vitro release of drugs from SLN

Apart from the in vitro release data presented in the above section, various other studies have been published with regard to potential parenteral application. Cavalli and coworkers prepared stealth and non-stealth Tripalmitin SLN loaded with Paclitaxel in order to provide an alternative for the parenteral administration (Crowe LM et al., 1986). The commercially available product Taxol® is a toxicologically critical micellar solution of the drug in Cremophor EL. Cavalli and coworkers reported sustained in vitro release: 0.1% of the Paclitaxel is released into the receptor medium (phosphate buffer, pH 7.4) after 120 min, this is correlated to first pseudo zero order kinetics. The same academic group has previously shown similarly sustained in vitro release profiles for doxorubicin and idarubicin (0.1% after 120 min) in contrast to burst release from reference solutions. For stearic acid SLN containing cyclosporin A, they determined an in vitro release of < 4% after 2 h compared to >60% from solution (Hauser H and Strauss G et al., 1988). Yang and coworkers determined the in vitro release of camptothecin from stearic acid SLN in conjunction with potential targeting to the brain using a dialysis bag technique at 37 °C. The data revealed a sustained release and could be fitted to a Weibull distribution ($t_{1/2}$ = 23.1 h). Heiati and coworkers studied the in vitro release of azido- deoxythymidine palmitate (AZT-P) from trilaurin SLN using a bulk-equilibrium reverse dialysis sac technique at 37 °C. The observed initial burst is attributed to partial AZT-P localization in phospholipids micelles. Further, a dependence of release profile on the type of phospholipid could be shown, i.e. phospholipids with phase transition temperatures (PTT) below 37°C lead to fast release, PTT >37°C represented a stronger diffusional barrier causing slower release.

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