CHAPTER II

LITERATURE REVIEW

1. AIDS

AIDS or acquired immunodeficiency syndrome is an infectious disease caused by human immunodeficiency virus (HIV). In the world, 5.2 per 100,000 populations have died with AIDS. In the year 2000, more than 36 million adults and children were living with HIV/AIDS. Forty seven percent of these are women. The cumulative number of HIV patients until 2003 was more than 60 millions (Fauci, 2003). In the year 2004, the people who were living with HIV/AIDS were enormously increased in number to approximately 42 million (Flexner, 2006). Nowadays, this disease cannot be cure. The effective antiretroviral therapy is needed to continue suppression of HIV replication resulting in slowing down the disease development to AIDS.

The first case of AIDS patient was found in 1981. Since that time, the disease has been extensively studied. Luc Montagnier isolated HIV-1 and published his results in 1983. In 1984, Robert C. Gallo and colleagues published the experiment data showing that HIV is the pathogenic agent that causes AIDS (Fauci, 2003). Then, the researches in AIDS field have extensively studied to find effective treatments for this disease.

1.1 HIV

HIV is in the family of retroviridae, in the genus of lentivirus. It replicates inside the host cells. The hosts for HIV are humans and chimpanzees. There are two major types of HIV, HIV-1 and HIV-2. HIV-1 is more worldwide whereas HIV-2 is mostly found in western Africa. The sensitivities of both HIV-1 and HIV-2 to most antiretroviral drugs in vitro are similar, however the non-nucleoside reverse

transcriptase inhibitors exhibit antiHIV activity against only HIV-1, not HIV-2 (Flexner, 2006).

HIV has spherical shape with size of approximately 100 nm in diameter. Two main compartments of the virus are envelope and capsid (core). The glycoprotein 41 and the glycoprotein 120 form the spike shape of the envelope. The envelope also contains other cellular protein and phospholipids membrane. The phospholipids are originated from the cell membrane of the host cell. Inside the envelope is the capsid that contains viral protein known as p24 and two copies of identical single strands of RNA. Each RNA strand bears important nine genes that are gag, pol, env, tat, rev, nef, vif, vpr, and vpu. Gag, pol, and env encode the structural protein, viral enzymes (reverse transcriptase, protease and integrase) and transmembranal envelope protein. While other genes such as tat, rev, nef, vif, vpr, and vpu encode the proteins that create new virus copies, regulate infectivity and combat host defences (Flexner, 2006). The structure of HIV is shown in figure 3.

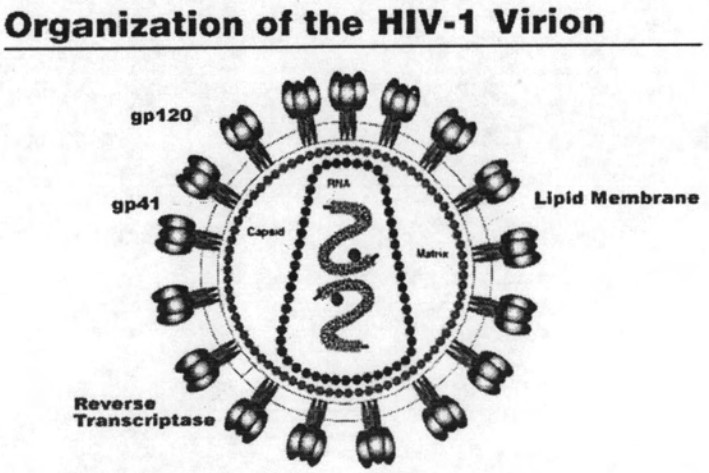


Figure 3 Structure of HIV (available from: http://www3.niaid.nih.gov/news/newsreleases/2004/organization_hiv1virion_fig.htm).

1.2 Transmission of HIV

The HIV infection can be transmitted through unprotected sexual intercourse, sharing injections in drug users, through contaminated needle sticks or blood products, semen, transmission from mother to fetus, and oral sex (rarely, but documented (Wilkin and Wilkin, 2002).

1.3 Life cycle of HIV

Target cells of HIV are cells in the immune system that contain CD4 receptors. HIV binds host cells on CD4 receptor. The binding also requires coreceptor on host cells (CCR-5 or CXCR-4) for fusion. The conformation change of viral glycoproteins (gp 41 and gp120) induced by CD4-coreceptor complex causes the close association between HIV and host cells resulting in fusion between viral envelope and the host cell's membrane. When HIV fuses with host cells, the HIV components are released into the host cell's cytoplasm. Subsequently, reverse transcriptase enzyme converts single strand of viral RNA to double strands of DNA. The double stranded DNA is then transported to the nucleus and integrated into host' DNA by integrase enzyme. The proviral DNA serves as a template to produce new viral RNA. The new viral RNA is exported to the cytoplasm where it is translated to the viral polyprotein. The HIV protease enzyme cut the large polyprotein precursors into various functional proteins. Finally, the viral genome and viral components are assembled that compile a new virus particle, following by budding from the host cell that has ability to infect other new host cells (Luber, 2005). The life cycle of HIV is shown in figure 4.

The main target cells of HIV are the cells containing CD4 receptors such as T lymphocytes, monocytes, macrophages, and B lymphocytes. HIV can also infect other cells such as central nervous system, retina elements, neuroendocrine cells of intestinal mucosa (Giammona et al., 1999).

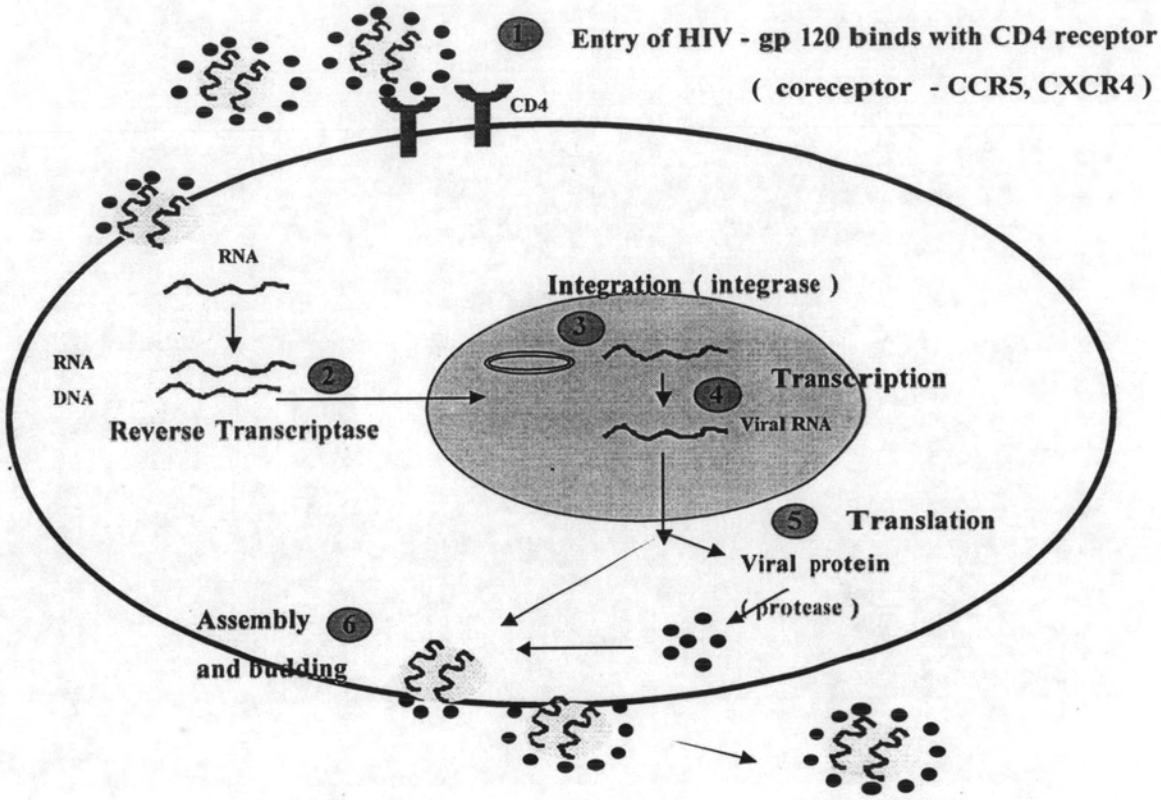


Figure 4 HIV life cycle (adapte from: http://www.open2.net/healthliving/health_socialcare/hiveffects_do.html).

1.4 The course of HIV infection

After HIV infection, the burst of HIV replication occurs rapidly that peaks at 2 to 4 weeks. In this period of primary infection, HIV patients may not reveal any symptoms or show non-specific symptoms such as rash, pharyngitis, adenopathy, fever, diarrhea, stiff neck, headache, fatigue, night sweats, arthralgia, myalgia. In this period, the number of CD4+ T cells decreases significantly and the level of the viral RNA copies in the plasma increase dramatically. The immune system responses to the infection by producing the CD8+ cytotoxic T cells and antibody leading to a decrease in viral replication and a recovery of CD4+ T cell number. In the clinical latency stage, the virus still continues replicate and the immune systems fight against the virus. When host immune system impairs, the virus replication is intense resulting

in high level of plasma HIV RNA. When the number of CD4+ T cells is lower than 200 cells/mm^3 resulting in high risk of opportunistic infections that lead to death. The symtoms of AIDS are herpes, tuberculosis, and oral lesions from fungi infection, lymphnode pathology and virus-induced cancers may be developed (Wilkin, T. and Wilkin, A., 2002, Flexner, 2006).

The time of disease development varies individually. Without any treatments, the average time of disease progression until death is 10 – 11 years. However after the AIDS symptoms appear, the patients normally die within 2 – 3 years (Fauci and Desrosiers, 1997). The time course of HIV infection is shown in figure 5.

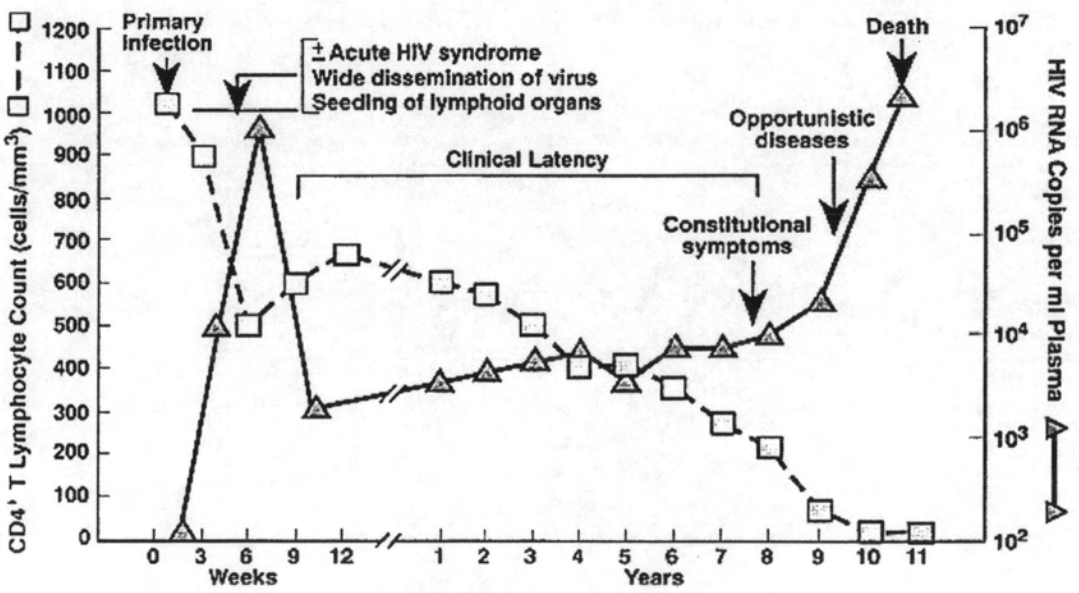


Figure 5 The course of HIV infection (Cohen and Fauci, 2001).

2. AntiHIV drugs

Most of antiHIV drugs are classified into three categories, reverse transcriptase inhibitors, protease inhibitors and entry inhibitor (Flexner, 2006).

2.1 Reverse transcriptase inhibitors

Reverse transcriptase inhibitors are the first antiHIV drugs. They interferes reverse transcriptase enzyme that is important for producing the new copies of viral genome. There are 2 types of the reverse transcriptase inhibitors that are nucleosides and non-nucleoside analogues.

2.1.1 Nucleoside reverse transcriptase inhibitors (NRTI)

The nucleoside reverse transcriptase inhibitors is the first class discovered antiHIV drugs. The drugs in this group are 2', 3'-dideoxynucleoside. The drugs must enter the cells and need to be fully phosphorylated at 5' OH to exhibit activity. They interfere reverse transcriptase enzyme by binding at the active site (substrate binding site) of the enzyme (Parang et al., 2000). The substrates for reverse transcriptase are nucleosides. Because the inhibitors have similar structure to normal nucleosides, so they compete the natural substrates to bind with reverse transcriptase and once the inhibitors are inserted into the DNA chain by reverse transcriptase. They cause chain termination because of the lack of 3' OH of the inhibitors, so DNA synthesis discontinues leading to interruption of HIV replication.

The first drug in this group is zidovudine that was initially developed unsuccessfully for use as anticancer drug. However, it is effective as antiHIV agent. Other drugs in this group are didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir, tenofovir, and emtricitabine.

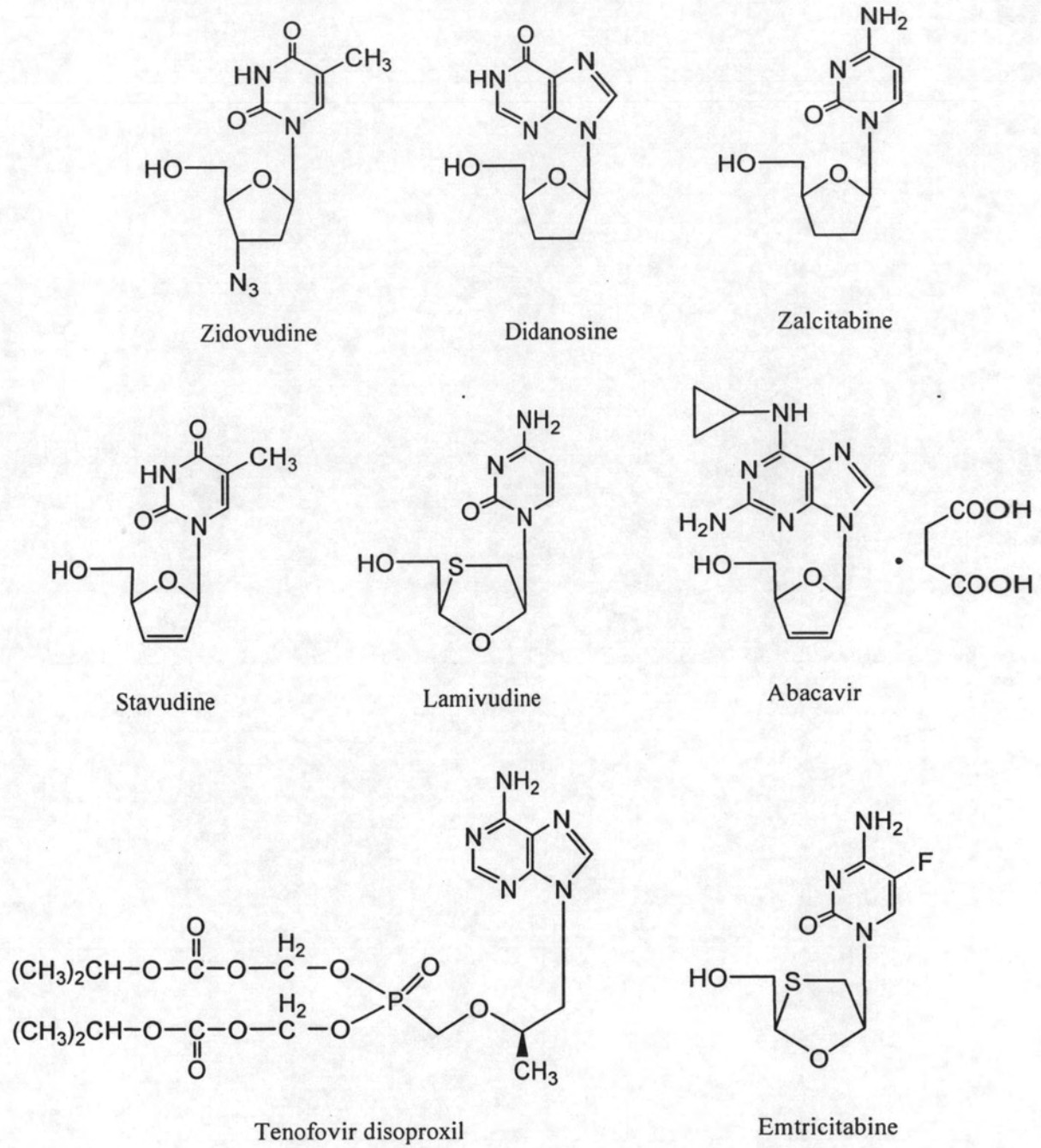


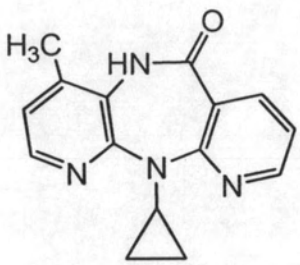
Figure 6 Chemical structures of nucleoside reverse transcriptase inhibitors (Clercq, 2001).

Table 1 Doses of nucleoside reverse transcriptase inhibitors (summarized from Clercq, 2001).

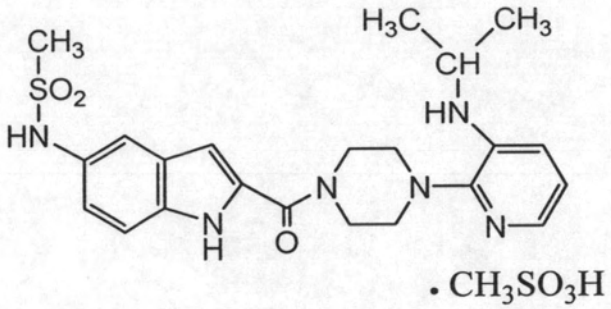
Drug	Other name	Dose	Regimens
Zidovudine	AZT	600 mg/day	devided to 2 -3 times a day
Didanosine	ddI	400 mg/day	200 mg twice a day 400 mg once a day
Zalcitabine	ddC	2.25 mg/day	0.75 mg 3 times a day
Stavudine	d4T	80 mg/day	40 mg twice a day
Lamivudine	3TC	300 mg/day	150 mg twice a day
Abacavir	Ziagen	600 mg/day	300 mg twice a day
Tenofovir	Viread	300 mg/ day	300 mg once a day
Emtricitabine	Coviracil	200 mg/ day	200 mg once a day

2.1.2 Non-nucleoside reverse transcriptase inhibitors (NNRTI)

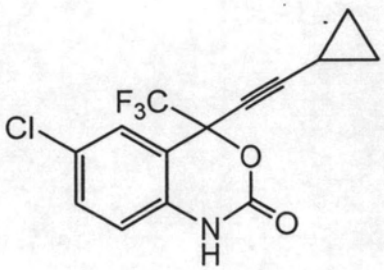
The non-nucleoside reverse transcriptase inhibitors have various structures. They interfere reverse transcriptase by binding at the site relative to the active sites of enzyme. The site that the inhibitors bind is not highly conservative; therefore, HIV develops the resistance to non-nucleoside reverse transcriptase inhibitors rapidly. This group of drugs is effective only with HIV-1. They have no activity against HIV-2 (Flexner, 2006).



Nevirapine



Delavirdine



Efavirenze

Figure 7 Chemical structures of non-nucleoside reverse transcriptase inhibitors (Clercq, 2001).

Table 2 Doses of non-nucleoside reverse transcriptase inhibitors (summarized from Clercq, 2001).

Drug	Other name	Total dose	Regimens
Nevirapine	Viramune	200 mg/day	200 mg once a day
Delvaridine	Rescriptor	1200 mg/day	400 mg 3 times a day (1 h apart from taking ddI)
Efavirenz	Sustiva	600 mg/ day	600 mg once a day (at bed time due to CNS side effects)

2.2 Protease inhibitors

Protease inhibitors interfere HIV protease enzyme in the latter stage of HIV life cycle. Normally, the HIV protease cut viral polyprotein precursors into smaller pieces of functional proteins at the Phe-Pro position. Instead of the Phe-Pro moiety, the protease inhibitors contain hydroxyethylamine moiety that cannot be cut by the HIV protease, so when they bind to protease enzyme, they inhibit the activity of the enzyme (Bragman, 1996, Kiso et al., 1999).

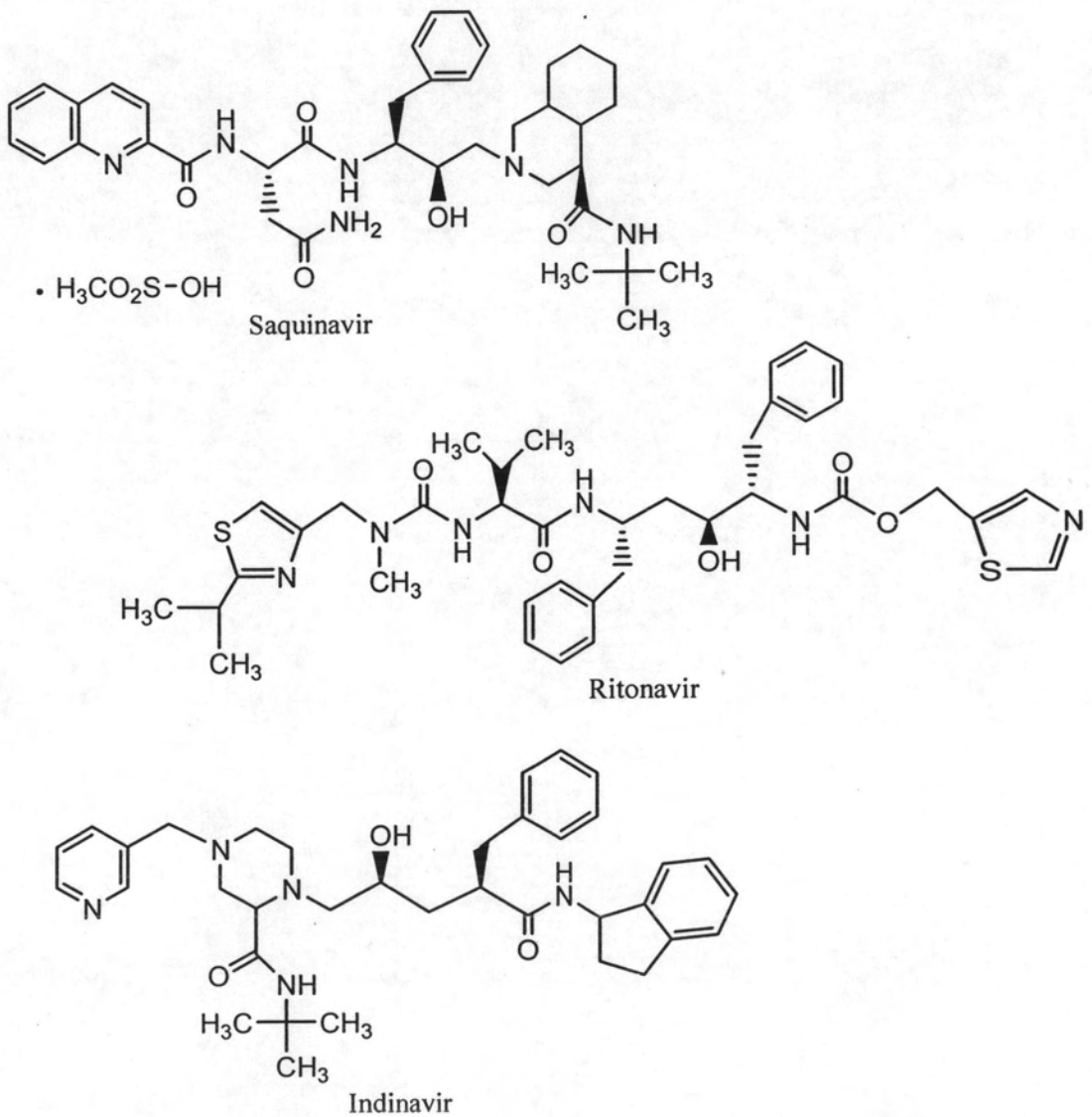


Figure 8 Chemical structures of protease inhibitors (Clercq, 2001).

Table 3 Doses of HIV protease inhibitors (summarized from Clercq, 2001).

Drug	Other name	Total dose	Regimens
Saquinavir	Invirase Fortovase	3600 mg/day	1200 mg three times a day (with food)
Ritonavir	Norvir	1200 mg/day	600 mg twice a day (with food)
Indinavir	Crixivan	2400 mg/day	800 mg three times a day (empty stomach)
Nelfinavir	Viracept	2250 mg/day	750 mg three times a day (with food)
Amprenivir	Agenerase Prozei	2,400 mg/day	1,200 mg twice a day (not with high fat food)
Lopinavir	Kaletra	1,000 mg/day	500 mg twice a day

2.3 Entry inhibitor

Only one drug in this class available is enfuvirtide (T-20) that is a synthetic HIV1-gp41- sequence derived peptide. Therefore it is effective only against HIV-1. It prevents membrane fusion and entry of the virus into the host cells by binding to CD4 receptor and CCR-5 coreceptor or CXCR-4 coreceptor (Luber, 2005). Enfuvirtide is reserved for patients who fail to use all other regimens. Route of administration of emfuvirtide is subcutaneous injection (twice a day in the dose of 100 mg/day) (Flexner, 2006).

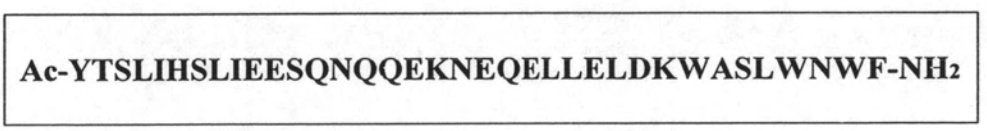


Figure 9 Chemical structure of entry inhibitor (Clercq, 2001).

3. HIV/AIDS therapy

Previously, when the nucleoside analogue inhibitors were discovered. This class of antiHIV drugs was used in monotherapy (as a single drug) and it was successful to suppress viral replication, but the problem occurred due to the virus developed drug resistance rapidly that limited the long-term clinical use of the drug. As a result, nowadays, multidrug therapy is recommended for HIV infection. The most common combination of this therapy is using at least 3 antiHIV drugs is known as the highly active antiretroviral therapy (HAART), which can be the combination of 2 drugs from NRTI plus 1 or 2 protease inhibitors or 2 drugs from NRTI plus a NNRTI or the third NRTI. The therapy increases the suppression efficiency to the viral replication causing more effectiveness and less resistance development. The treatments of HAART have been successful that change the perspective of the HIV infection from lethal disease to manageable disease (Cohen and Fauci, 2001, Luber, 2005, Verweij-van Wissen et al., 2005).

3.1 The problems of current therapy

Even the current therapy is successful to control the HIV infection in patients. Those drugs could not cure the HIV infection. Still the virus is not totally eliminated by current approaches. Side effects of the drugs result in discontinuing the drugs and also may be more severe when taken with other drugs. Toxicity from long-term use and development of drug resistance are limited the continuing treatments (Greene and Peterlin, 2002, Luber, 2005). Also, the treatment contains many drugs that need to be taken continuously for very long time that need high patients' compliance. Therefore, it is needed to develop better medication.

Table 4 The effects of co-administration of antiHIV drugs.

Drugs	Zidovudine	Zalcitabine	Didanosine	Stavudine	Lamivudine
Zidovudine		-additive effect -not help to slow the occurrence of drug resistance			
Zalcitabine			overlap S/E	overlap S/E	antagonist
Didanosine		overlap S/E		overlap S/E	
Stavudine	antagonist	overlap S/E	overlap S/E		
Lamivudine		antagonist			

Therefore, more effective antiretroviral therapy is needed to maintain the viral suppression in long term leading to slow down the progression of the disease, resulting in a longer lifespan and a better quality of life.

There are various strategies to improve the effectiveness of drugs.

1) synthesizing new compounds which have better activity
2) developing new pharmaceutical dosage forms
3) using new concept of therapeutics. i.e. making polymer-drug conjugates

In this study, the selected strategy is polymer-drug conjugates and selected model antiHIV drug is zidovudine.

4. Zidovudine

Zidovudine (3'-azido-3'-deoxythymidine or azidothymidine, AZT) was approved in 1987 and was the first antiHIV drug in the market. It is an antiHIV drug in the class of nucleoside reverse transcriptase inhibitors. It is an analogue of thymidine that possesses antiviral activity against retroviruses such as human T lymphotropic virus, HIV-1, HIV-2 (Hayden, 1996, Flexner, 2006). Its structure differs from thymidine that instead of the hydroxyl group, zidovudine contains an unreactive azido group at 3' position of the sugar (deoxyribose) ring (Parang et al., 2000). It's triphosphate form inhibits the reverse transcriptase which is the HIV's enzyme leading to the interruption of viral gene synthesis and the incorporation into the DNA chain causing the DNA chain termination (Kawaguchi et al., 1990, Meier et al., 1998, D'Alessandro et al., 2000, Thomas and Panchagnula, 2003). It is successful to slow down the progression of HIV infection to AIDS and it has been used to suppress the transmission of HIV from mother to fetus. AZT is commonly used in most antiHIV combination therapies for example the combination of AZT, lamivudine and indinavir, the combination of AZT, lamivudine and ritonavir or the combination of AZT, didanosine and nevirapine. The zidovudine treated therapy slows down the disease progression and improve survival rate (Parang et al., 2000, Chitnis et al., 2002).

4.1 Chemical properties

Zidovudine is crystalline solid, white to off-white and odourless. Its chemical name is 3'-azido-3'-deoxythymidine or 3'-azido- 2', 3'-dideoxythymidine. The molecular formula of AZT is $C_{10}H_{13}N_5O_4$ and the molecular weight is 267.24. Its melting point is 119-121 °C. The water solubility is 25 mg/ml at 25 °C (Sethi, 1991). The chemical structure of AZT is shown in figure 10.

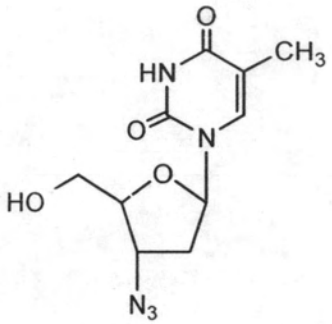


Figure 10 Chemical structure of zidovudine (Clercq, 2001).

4.2 Pharmacokinetics and metabolism of zidovudine

Zidovudine is rapidly absorbed after oral administration. The oral bioavailability is approximately 60-70%. For the oral dose of 100 mg every 4 h, the peak and trough for the drug concentration in plasma are 0.4 to 0.5 μg/mL and 0.1 μg/mL, respectively. Although zidovudine has a good oral bioavailability, it has short plasma half-life, which is approximately 0.9 to 1.5 hours. Three important metabolism pathway of AZT are AZT glucuronidation, AMT formation, and AZT phosphorylation. For AZT glucuronidation, the major metabolism pathway, AZT is rapidly transformed to 5'-O-glucuronide metabolite (5'-glucuronyl zidovudine or 3'-azido-3'-deoxy-5' β-D-glucopyranurosylthymidine) by UDP-glucuronyl transferase (UDPGT). The AZT glucuronidation pathway is dominant metabolism pathway of AZT and this pathway occurs in human liver microsome. The 5'-O-glucuronide metabolite plasma level is 2-5 times higher than AZT plasma level. Glucuronide metabolite has half-life approximately 1 h but it has no antiHIV activity (Veal and Back, 1995).

Other metabolism pathway is transformation of 3'-amino-3'-deoxythymidine in human liver. The 3'-amino-3'-deoxythymidine metabolite which is present at low concentration (about 10-15 % of zidovudine plasma concentration)

may be responsible for myelotoxicity of AZT-treated patients due to the evidence that AMT was 5-7 times more toxic than AZT on human haematopoietic progenitor cells. AMT has half-life of approximately 2.7 h (Veal and Back, 1995).

The key metabolism pathway for exerting antiHIV activity of AZT is AZT phosphorylation but it is a minor pathway that is about 1% of all metabolism pathways. The step of phosphorylation is shown in figure 11. AZT is phosphorylated to monophosphate form by thymidine kinase and to diphosphate form by thymidylate kinase that is the rate-limiting step in the AZT phosphorylation and finally to triphosphate form by pyrimidine nucleoside diphosphate kinase. The phosphorylated form of AZT has longer half-life than the parent drug (Veal and Back, 1995, Parang et al., 2000).

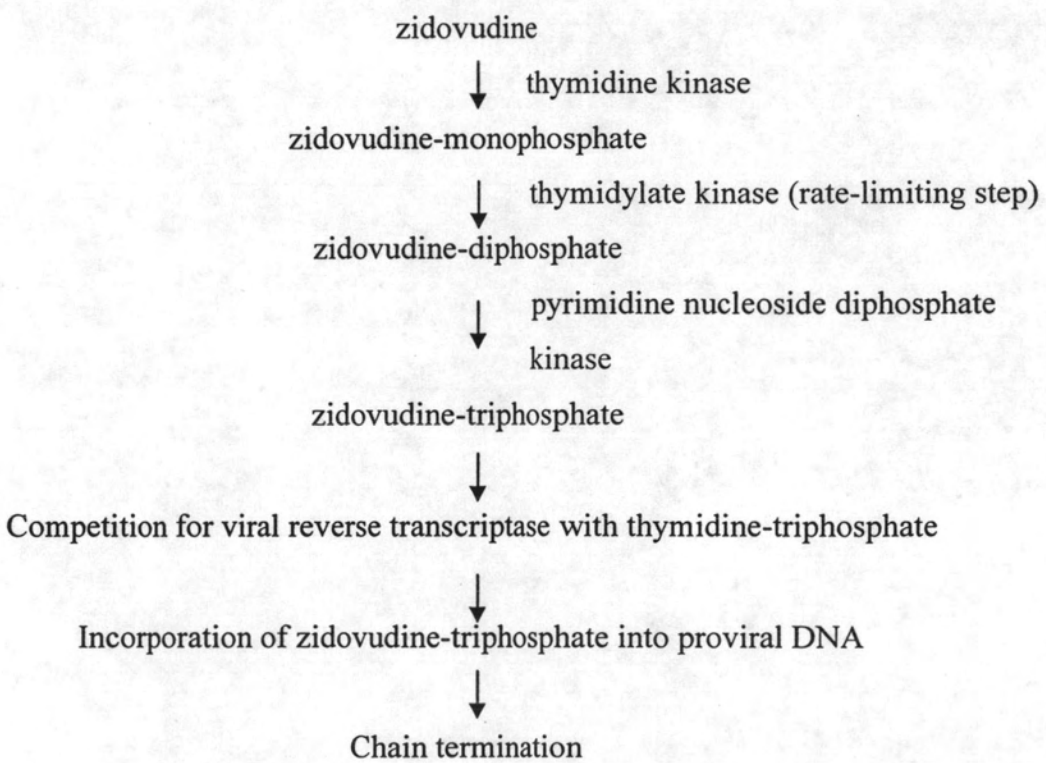


Figure 11 Mechanism of action of zidovudine.

The renal excretion in the form of parent drug is approximately 14 %, and that of its glucuronide metabolite is approximately 75% indicating that the body eliminates AZT by mainly transformation to glucuronide metabolite to be excreted by renal route (Hayden, 1996).

Coadministrion of AZT with didanosine (ddI) increases area under the plasma concentration time curve (AUC) of AZT and glucuronide metabolite significantly that may be due to changing the AZT absorption and/or decreasing renal elimination (Veal and Back, 1995).

It is necessary to maintain an adequate zidovudine concentration in the body to achieve the antiHIV effect. Due to the short plasma half-life of zidovudine and the transformation of parent drug to its inactive metabolites, the sustained release dosage forms of zidovudine should be developed.

4.3 Development of zidovudine dosage forms or prodrugs

A wide variety of drug delivery systems have been developed to improve the pharmacological properties of zidovudine, for example;

4.3.1 Liposomes

Liposomes loaded with zidovudine were prepared to improve the pharmacokinetic properties of zidovudine by coating the surface of zidovudine-loaded liposomes with targeting groups of palmitoylgalactoses. Zidovudine-loaded liposomes were prepared from the lipid components of egg phosphatidylcholine, cholesterol and phosphadtidylethanolamine in the ratio of 7:2:1, subsequently coated with palmitoylgalactose. The palmitoylgalactosyl zidovudine-loaded liposomes had particle size of 136.96 nm and could encapsulate zidovudine in the vesicles of 53.91 %. The palmitoylgalactosyl zidovudine-loaded liposomes released drug following the zero order release pattern in the manner of prolonged release. The coating of the liposomes with palmitoylgalactoses may provide the benefit for antiHIV agents by

enhancing the targeting efficiency of these particular liposomes to cells containing lectin receptors such as monocytes, macrophages and lymphocytes that are the target cells of HIV. The researchers performed the cellular drug uptake study by determining zidovudine levels inside rat alveolar macrophages. Cellular drug uptake from the palmitoylgalactosyl zidovudine-loaded liposomes was greater than that from uncoated zidovudine-loaded liposomes and that from free zidovudine indicating the enhancement of cellular uptake by specific coating liposomes with palmitoylgalactose. The *in vivo* study in rats showed an increase in zidovudine half-life to 20.77 h from uncoated zidovudine-loaded liposomes and to 73.09 h from palmitoylgalactosyl zidovudine-loaded liposomes (Garg and Jain, 2006).

4.3.2 Niosomes

Zidovudine-entrapped niosomes were prepared by nonionic surfactants of polysorbate-80 and polyglyceryl-3-diisosterate in which bilayer of these niosomes were stabilized by myristyl alcohol. Zidovudine entrapment in niosomes was 85 %. The *in vitro* release study showed that the niosomes could release zidovudine about 20 % within 18 h. The pharmacokinetic study of zidovudine-entrapped niosomes performed in rabbit following intravenous bolus administration showed higher zidovudine levels and sustained levels obtained from zidovudine-entrapped niosomes than that obtained from zidovudine solution. The niosomes had increased half-life, reduced clearance, increased area under the concentration-time curve (AUC) and increased area under the first moment of the concentration-time curve (AUMC), and increased mean residence time (MRT) (Gopinath et al., 2001).

4.3.3 Nanoparticles

Zidovudine-entrapped nanospheres in submicron sizes were prepared for sustained release dosage forms. The zidovudine-entrapped nanospheres were performed using biodegradable polylactide-co-glycolide in the ratio of 50:50 and the product appeared as white powder. Zidovudine entrapment was found to be 19.4 % w. The solution of zidovudine-entrapped nanospheres in normal saline was co-

administered orally with mucoadhesive gel as an adjuvant in order to improve bioavailability by enhancement the adhesion of the zidovudine-entrapped nanospheres to the surface of intestinal epithelium. The mucoadhesive gel contained carbopol-934-P, metho-6, sodium hydroxide, orange oil, syrup, glycerin, yellow lake no. 6 and water. The pharmacokinetic studies of zidovudine-entrapped nanospheres with or without mucoadhesive gel were conducted in rabbits compared to that of oral zidovudine syrup and that of zidovudine i.v. solution. The results showed that after the administration of zidovudine solution (10 mg/kg body weight) intravenously, zidovudine was eliminated from plasma rapidly. Oral administration of zidovudine syrup (in the dose of 10 mg/kg body weight) showed time to peak (t_{max}) of 1 h and peak concentration of 3.6 μM. The oral co-administration of zidovudine-entrapped nanospheres in the dose of 50 mg/kg body weight with mucoadhesive gel provided sustained release of zidovudine in the plasma with higher bioavailability compared with that obtained from zidovudine-entrapped nanospheres without mucoadhesive gel indicating the enhancement effect obtained from mucoadhesive gels that help to slow the absorption of zidovudine from gastrointestinal tract (Callender et al., 1999).

Other research studied the targeting of zidovudine to the gut associated lymphoid tissues in the gastrointestinal mucosa because lymphoid tissues containing many immune cells are the HIV reservoir. The targeting procedure was by delivering zidovudine-loaded nanospheres made from poly(isohexylcyanoacrylate) to the gastrointestinal tract. Zidovudine loading in the nanospheres was approximately 8 % w. The zidovudine-loaded nanospheres was 250 nm in diameter. *In vitro* drug release studies were performed in simulated gastric juice and intestinal media showing that zidovudine was released in burst release manner and was released more progressive in the presence of esterase enzymes. The *in vivo* determination of zidovudine concentration in intestine was performed in three rats that received zidovudine in the dose of 2.5 mg/kg body weight as colloidal suspension of nanoparticles. The nanoparticles were concentrated in the gastrointestinal tract at least 4.4 times at 30 min and 5.9 times at 90 min higher than that found from the control solution of zidovudine. The nanoparticles were localized in the gastrointestinal tract for prolonged period whereas zidovudine concentration was low

in the blood and other organs. The zidovudine concentration from nanoparticles were concentrated in the Payer's patches 4 times higher than that from zidovudine solution whereas the amount of zidovudine that reached the lymphatic flow was lower in the case of the nanoparticles when compared with zidovudine solution (Dembri et al., 2001).

4.3.4 Microspheres

Zidovudine-loaded microsheres were prepared using ethyl cellulose with the ratio of drug to polymer of 0.5 (formula I) and 0.9 (formula II). The *in vivo* release study in Beagle dogs after oral administration revealed that zidovudine-loaded microspheres formula I could prolong zidovudine level better than formula II and formula II was better than zidovudine power. The results showed that zidovudine release from formala II was almost complete within 5 h whereas that from formula I was sustained for 8 h with higher plasma zidovudine level. The higher drug loading (higher drug to polymer ratio) caused higher porosity of polymer matrix resulting in faster release manner (Abu-Izza et al., 1997).

4.3.5 Extended-release tablets

Zidovudine extended-release tablets were prepared using hydrophilic Eudragit RLPO and RSPO or with hydrophobic ethyl cellulose. The tablets were prepared by wet granulation and being compressed into tablets. *In vitro* drug release study was performed in hydrochloric acid for 3 h and subsequently performed in phosphate buffered saline pH 7.4 until 12 h. The tablet containing Eudragit could sustain release the drug for 6 h, whereas the tablet containing Eudragit and ethyl cellulose could sustain release the drug for 12 h due to the hydrophobicity of ethyl cellulose, which restricts the drug diffusion through the matrix. The combination with ethyl cellulose reduced the burst release obtained from using Eudragit alone. The *in vivo* study in rabbits showed that the administration of oral tablets containing Eudragit and ethyl cellulose provided maintained zidovudine level in plasma up to 12 h (Kuksal et al., 2006).

4.3.6 Polymer-zidovudine conjugate

The only one type of polymer-zidovudine conjugate has been prepared. The polymer-zidovudine conjugate was synthesized by Giammona' research group (Giammona et al., 1998) using α, β–poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) polymer, a synthetic protein-like structured polymer containing positive charge. The drug loading in PHEA-zidovudine conjugate was 7.4 % w/w. The *in vitro* drug release study was carried out in various buffer solutions and in human plasma. In buffer pH 1.1, PHEA-zidovudine conjugate released low amount of zidovudine (< 3.5 %) and succinyl-zidovudine (approximately 2 %) after 6 h. In buffer pH 5.5, approximately 3 % of zidovudine was released from the conjugate after 24 h whereas succinyl-zidovudine was not detected. In buffer pH 8, PHEA-zidovudine conjugate released zidovudine of less than 20 % and succinyl-zidovudine of about 5 %, whereas at the same pH with α-chymotrypsin, the conjugate released 45 % of zidovudine and about 5 % of succinyl-zidovudine after 24 h. The release study in pH 7.4 showed that 13.5 % of zidovudine and 5.5 % of succinyl-zidovudine were released from the conjugate after 24 h. The *in vitro* release study in plasma showed that higher amount of zidovudine (> 60 %) and succinyl-zidovudine (11 %) were released from the conjugate in human plasma within 24 h. The *in vitro* data indicated that PHEA-zidovudine conjugate could prolong the release of the drug.

5. Polymer therapeutics

Polymer therapeutics is a term that includes polymeric drug, polymeric micelles, polymer-protein conjugate, polymer-drug conjugate and multi-component polyplexes that contain water-soluble polymers as bioactive polymer or as inert polymer for drug carrier (figure 12). They differ from conventional drug delivery system that they are involved in chemical conjugation. Polymer-protein conjugates are already in the market, PEG-adenosine deaminase, PEG-asparaginase and styrene maleic anhydride-neocarzinstatin. Polymer-drug conjugate for anticancer drug is in clinical trial. Because of promising polymer-anticancer conjugate and polymer-

protein conjugate, the field of polymer therapeutics is getting interest in nanotechnology (Duncan, 2003).

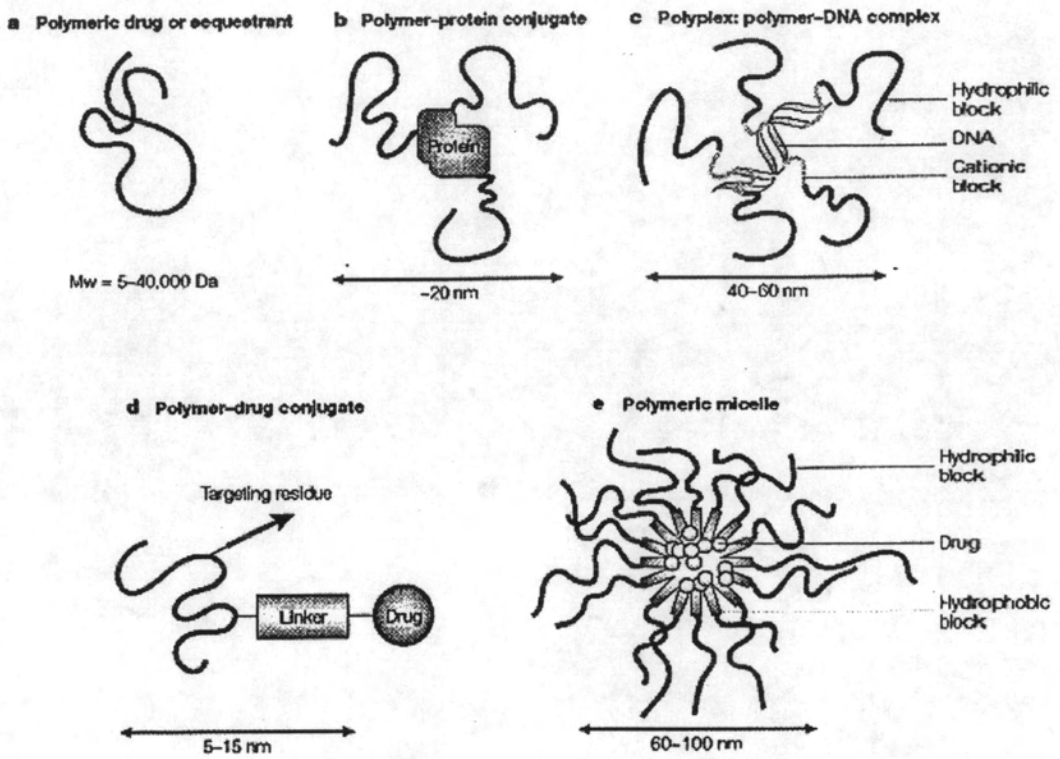


Figure 12 Schematic representation of polymer therapeutics; polymeric drug (a), polymer-protein conjugate (b), polyplex (c), polymer-drug conjugate (d) and polymeric micelle (e) (Duncan, 2003).

5.1 Polymer-drug conjugate

Polymer-drug conjugate can be considered as one type of macromolecular prodrug. The prodrug can be described as a pharmacologically inactive form of parent drug that is able to release free drug within the body by chemical and/or enzymatic transformation (Giammona, Cavallaro and Pitarresi, 1999). Many drugs were linked to polymers in order to achieve better efficiency of drugs since in the early of 1950s. In that period, polymer chemists focused on the chemistry aspects more than the biological aspects. The first model of pharmacological active polymer (polymer conjugate's concept) was proposed by Ringsdorf in 1975. The proposed model of polymer conjugate is composed of five components that are polymer, drug, spacer, targeting group and solubilising agent (Hoste et al., 2004).

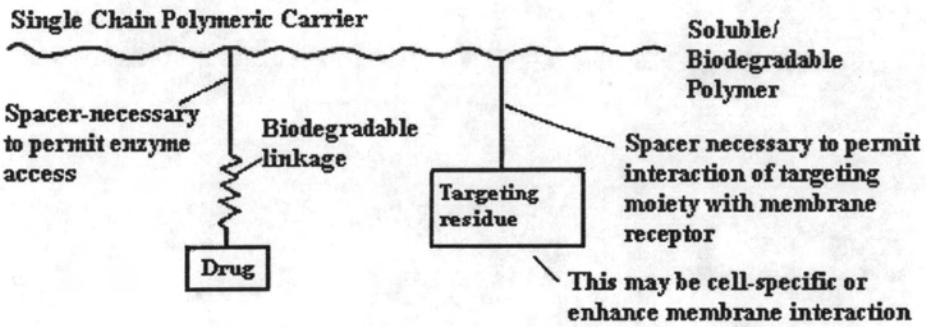


Figure 13 Model of polymer-drug conjugates (Dumitriu and Dumitriu, 1994).

5.2 The benefits of polymer-drug conjugates

Polymer-drug conjugates can be designed to

1) prolong drug release, prolong pharmacological activity of drug or duration of action
2) reduce toxicity or adverse effects of drug
3) increase solubility
4) increase stability
5) improve site-specificity (target to specific site)
6) increase patient acceptance (Zovko et al., 2001)
7) alter pharmacokinetics (Dumitriu and Dumitriu, 1994).

5.3 The characteristics of polymer-drug conjugates

1) The polymer is biocompatible and nonimmunogenic.
2) The polymer is biodegradable or has low molecular weight to be eliminated by renal route.
3) The polymer contains functionality for covalently linking to a bioactive agent via a linker that may be cleaved by hydrolysis or enzymatic cleavage.
4) A spacer may be used to separate the polymer backbone and the drug that can be designed to control the rate of release of linked drug, improve the drug-receptor interaction or provide selectivity of the linker to be cleaved by some enzymes or cleaved at specific site.
5) Solubilizing agents may be attached as additional pendant groups for regulation of solubility.
6) A drug targeting group or ligand may be attached for cell specific recognition that can alter cellular uptake by specific cell types resulting in an increase in the efficacy of drugs and a reduction in toxicities (Dumitriu and Dumitriu, 1994, Zovko et al., 2001, Hoste et al., 2004).

6. Dextrin as polymeric drug carrier

Dextrin is natural polymer that is obtained from corn starch via the process of enzymatic hydrolysis. Dextrin is α-1,4 poly(glucose) polymer. It contains more than 95% of α-1,4 links and less than 5% of α-1,6 links. Dextrin contains linear chains composed of glucose units that are connected by α-1,4 glycosidic linkages. Many linear chains are attached each other with α-1,6 glycosidic linkages. Dextrin is water soluble and biodegradable polymer. It is readily degraded by α-amylase to maltose and isomaltose. In clinical application, dextrin (Icodextrin; a polydisperse dextrin) has been used in a peritoneal dialysis. It is non-toxic and well proven for non-immunogenicity. Also Icodextrin has been developed as a carrier for intraperitoneal injection of 5-fluorouracil (Hreczuk-Hirst, German and Duncan, 2001, Hreczuk-Hirst et al., 2001). The chemical structure of dextrin is shown in figure 14.

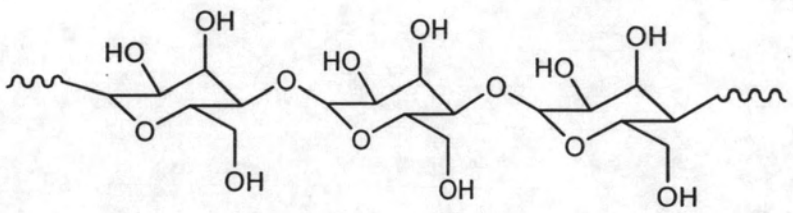


Figure 14 Chemical structure of dextrin.

6.1 Dextrin-drug conjugates

Dextrin has been conjugated to doxorubicin (anticancer drug) and amphotericin B (antifungal drug) as described below.

6.1.1 Dextrin-doxorubicin conjugate

Dextrin-doxorubicin conjugate was synthesized by Duncan's research group. Doxorubicin is an anticancer drug that has very low water solubility of about 1 mg/ml. The drug loading on dextrin-doxorubicin conjugate was

approximately 9 % w. Resulting dextrin-doxorubicin conjugate could increase solubility of doxorubicin approximately 4 times. The dextrin-doxorubicin conjugate could be soluble in water up to 40 mg/ml or equivalent to doxorubicin of 4 mg/ml (Hreczuk-Hirst, German and Duncan, 2001).

6.2.2 Dextrin-amphotericin B conjugate

Amphotericin B is anti-fungal agent but it has very low water solubility and toxicity. Therefore, the dextrin-amphotericin B conjugate was synthesized in order to increase water-solubility and improve drug targeting. The drug loading of the dextrin-amphotericin B conjugate was about 0.6 – 16.0 % w that varied upon to degree of succinylation of dextrin (availability of free carboxylic groups). The dextrin-amphotericin B conjugate was water-soluble (> 0.4 mg/ml) that was higher than free amphotericin B (22 μg/ml). The hemolysis study in red blood cells showed that dextrin-amphotericin B conjugate had much higher concentration (< 50 μg/ml) that make 50 % hemoglobin release from red blood cells than that of free drug (5.5 μg/ml) (German et al., 2000).