CHAPTER 2
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Liposomes

2.1.1 Introduction

Liposomes are spherical vesicles composed of lipid bilayers arranged around a central aqueous core. They can be composed of phospholipids, liposomes have the ability to incorporate lipophilic and amphiphilic drugs within their phospholipid membrane or they can encapsulate hydrophilic compounds within the aqueous core as shown in Figure 1.2. Liposome formulations can therefore increase safety and efficiency of drugs in bio-environment and could also help in site specific drug delivery (Brandl, 2001; Massing and Fuxius, 2000; New, 1990).

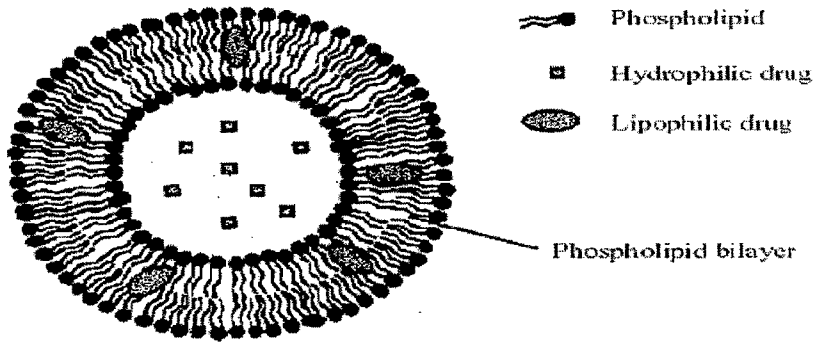

Figure 2.1 A schematic representation of incorporation and encapsulation of drugs into a liposome (Massing and Fuxius, 2000)

2.1.2 Liposome composition

A phospholipid exists of a hydrophilic head group and lipophilic tails. The polar head can be charged or uncharged and the lipophilic tails are composed of fatty acids chains (Figure 2.2).

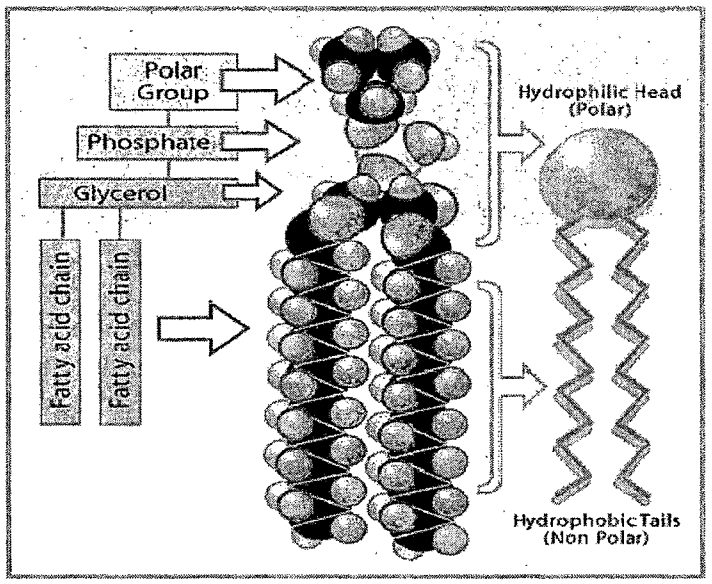

Figure 2.2 A schematic representation of a phospholipid (http://www.bioteach. ubc.ca/Bio-industry/Inex/)

2.1.3 Relevance of liposome size

The sizes of liposomes can be divided into large, intermediate and small and display different features depending on their size (Brandl, 2001). Even though large liposomes have the largest entrapped volume they are not ideal for intravenous administration since they are too big for escaping the macrophages during circulation in the blood pool. Intermediate liposomes can escape macrophages and stay in the blood pool long enough for reaching targets close to the circulation. Small liposomes have the smallest captured volume and a shorter circulation time compared to intermediate liposomes due to capillary extravasation. However, this gives them the unique ability to reach targets outside the blood pool such as solid tumors (Brandl, 2001; New, 1990). One possible classification according to size is listed in Table 1.1.

Table 2.1 Classification of liposomes according to size

Liposomes Classification	Size (nm)	No. of lamellae
Multilamellar vesicles	100-1000 nm	>5
Small unilamellar vesicles	<200 nm, Lowest possible size	1
Large unilamellar vesicles	>200 nm	1

Liposomes can change in shape during storage of dispersions. There are two different types of alterations increasing the size of liposomes, aggregation and fusion (Brandl, 2001). This alters the liposomal features. Aggregation is a process where liposomes are linked together without breaking the membranes. This process is reversible. However, fusion is an irreversible process, where liposome membranes are broken and melted together with other liposomes forming bigger particles. An increase in size is thus a direct result of a change in shape.

2.1.4 The rationale for using liposomes in chemotherapy

Intravenous route of administration is considered as the most promising route for liposomal formulation. The role of the liposome (containing the drug) is to circulate in the blood in order to reach the desired organ or tissue. The liposomal membrane acts as a barrier protecting the drug from premature elimination or metabolism. At the same time, the liposome membrane is controlling the release of the cytotoxic agent. The liposome carrier may also direct drugs to the tumor site. Thereby, the therapeutic window and toxicity profile of drug compounds can be improved (Brandl, 2001). The liposomal accumulation at tumor site is referred to as the enhanced permeability and retention effect and is based on dissimilarities of healthy and cancerous tissues. The endothelial walls of blood vessels in tumors are leakier than those in healthy tissues because of an increased number of bigger gaps. Thus, small liposomes are able to extravasate and penetrate into solid tumors. At the same time, the liposomes stay longer within the tumor site since the removal by the lymphatic system is greatly reduced in cancerous tissue. These special characteristics of cancerous tissue facilitate an accumulation and retention of small liposomes at tumor site, respectively shown in figure 2.3 (Brandl, 2001; Massing and Fuxius, 2000).

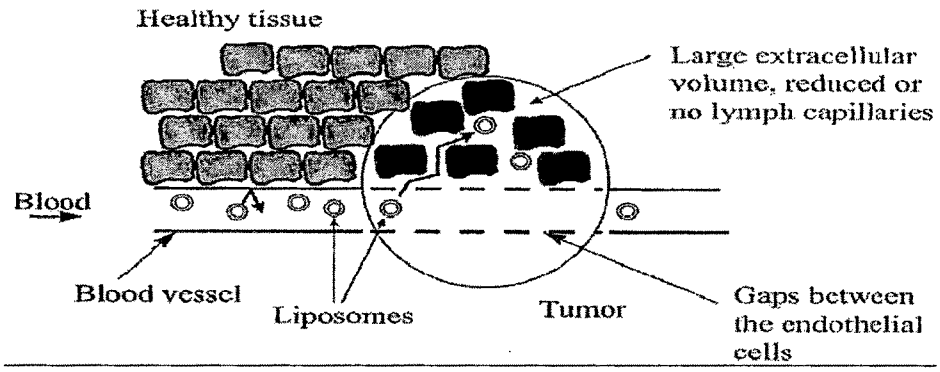

Figure 2.3 The enhanced permeability and retention effect (Massing and Fuxius, 2000)

2.1.5 Challenges with liposome formulations

Upon entering the general circulation the liposomes must be able to avoid uptake by macrophages. Once plasma proteins adsorb to the liposomal surface the liposomes are easily taken up by macrophages resulting in a decrease in liposomal elimination half life ($t_{½}$). Adsorption of plasma proteins to liposomes can be reduced by producing small liposomes with rigid uncharged membranes, such as HSPC and cholesterol. The stability of liposomes can also be affected prior to administrating the drug. The shelf life of the product is dependant upon the chemical and physical stability. The different components in the final liposomal product can interact with each other resulting in degradation. In addition, hydrophilic drugs with a low molecular weight (MW) are prone to diffuse through the liposomal membrane, reducing the shelf life of the product. In this case a tightly packed membrane is essential. Thus, the liposomal membrane composition, i.e. lipid composition and lamellarity is important since it together with the physiochemical characteristics of the drug determines the retention of the active ingredient within the liposome (Brandl, 2001; Massing, 2000).

2.2. Gemcitabine

The drug replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. The process arrests tumor growth, as new nucleosides cannot be attached to the "faulty" nucleoside, resulting in apoptosis (cellular "suicide"). They prevent these substances becoming incorporated in to DNA during the "S" phase (or DNA synthesis phase of the cell cycle), stopping normal development and division. Gemcitabine HCl blocks an enzyme which converts the cytosine nucleotide into the deoxy derivative. In addition, DNA synthesis is further inhibited because gemcitabine HCl blocks the incorporation of the thymidine nucleotide into the DNA strand.

2.2.1 Therapeutic indications

Table 2.2 Therapeutic indications of Gemcitabine

Cancer Type	Combination	Stage in Cancer
Breast cance-2nd line	Paclitaxel	Metastatic
NSCLC-1st line	Cisplatin	IIIA & IIIB (inoperable locally advanced), IV (metastatic)
NSCLC-palliative treatment	NIL	
Pancreatic Cancer: 1st and 2nd line*	NIL	II & III (inoperable locally advanced), IV (metastatic)
Bladder Cancer	Cisplatin	IV (Muscle invasive)
Epithelial Ovarian Cancer	Carboplatin	III (Locally advanced), IV (metastatic)

* for patients previously treated with 5-FU

2.2.2 Mechanism of action

The prodrug gemcitabine is converted intracellularly via deoxycytidine kinase to difluoro deoxycytidine monophosphate, which is further converted to two active metabolites, dFdCDP and dFdCTP, di– and triphosphate, respectively. Firstly, dFdCDP inhibits the catalysing enzyme ribonucleotide reductase resulting in a reduced amount of deoxynucleotide, deoxycytidine triphosphate (dCTP), available for DNA synthesis. Secondly, dFdCTP competes with dCTP for incorporation into DNA. Incorporating dFdCTP results in chain termination after the further addition of one more nucleotide and thus to apoptosis. Thus dFdC affects the synthesis phase of cell

metabolism in two different ways and exhibits a self potentiating effect (Noble and Goa, 1997; http://www.cancer.gov/Templates/drugdictionary.aspx?CdrID=41213).

2.2.3 Pharmacokinetics

After intravenous doses gemcitabine is rapidly cleared from the blood and metabolised by cytidine deaminase in the liver, kidney, blood and other tissues. Clearance is about 25% lower in women than in men. Almost the entire dose is excreted in urine as 2′-deoxy-2′,2′-difluorouridine (dFdU), only about 1% being found in the faeces. Intracellular metabolism produces mono-, di- and triphosphate metabolites, the latter two are active. The half-life of gemcitabine ranges from 32 to 94 minutes depending on age and gender. The intracellular half life of the triphosphate is stated to range from 0.7 to 12 hours (Johnson, 2000; Shamseddine et al., 2005).

2.2.4 Toxicity

The cytotoxic activity of gemcitabine *in vivo* is dose and dosage regimen dependent (Noble and Goa, 1997; Moog, 2002). This means the activity and the toxicity are related to the dose given and the dosage interval of the treatment. The problem with gemcitabine is its short plasma $t_{½}$ and its quick metabolism into dFdU followed by elimination from the body. Therefore high doses of dFdC are required in order to achieve sufficient cytotoxic concentrations of dFdCTP (Moog, 2002). Due to the narrow therapeutic window, high administered doses increase the possibility of toxicities and concentration dependent side effects for patients. According to clinical studies the primary dose limiting toxic effect is myelosuppression, neutropenia, leucopenia, anaemia and thrombocytopenia. In addition, together with other side effects such as hepatic abnormalities, nausea and vomiting, 10 % of patients ceased treatment (Noble and Goa, 1997).

2.2.5 Prevention of problem associated with drug

A high amount of dFdC in the aqueous compartment of liposomes with a negligible amount of dFdC outside the liposomes could increase the potency and decrease the toxicity associated with gemcitabine treatment.

2.3 Folate Delivery

2.3.1 Introduction

Current efforts to selectively deliver chemotherapeutic agents to their sites of action have focused on exploiting the natural endocytosis pathway by targeting an over-expressed receptor or antigen on the surface of target cell. Receptor-mediated targeting of long circulating carriers has been accomplished by attachment of ligands to the distal end of the polymer chains. Interaction between the targeting ligands and the cell surface receptors allows the carrier to be bound to the cell surface for a time sufficient to facilitate internalization of the vehicle and the drug that it contains. A number of ligand-presenting delivery systems targeting differentially expressed receptors on tumor cells have been studied to promote increased drug uptake (Eavarone et al., 2000; Gabizon et al., 1999; Kurihara et al., 1999; Lappi, 1995; Lee and Low, 1995).

2.3.2 Internalization of folate-bearing drug carriers

Ligand-bearing drug carriers enter the cell through receptor-mediated endocytosis, a process which involves binding of ligand-bearing carriers with receptors expressed on the surface of the cell followed by internalization of the complex. Lipid rafts represent the major pathway for internalization of many glycosyl phosphatidylinositol-anchored (GPI) proteins. The rafts are normally 50-300 nm in size and comprise a minor fraction of the cell membrane (Jacobson et al., 1999; Mayor and Varma, 1998; Sheets et al., 1997). Folate receptor, a GPI-anchored protein over-expressed on a number of cancer cells is internalized through this pathway. Several studies have shown that GPI anchored proteins are found to be clustered in specialized lipid rafts called microdomains. It has been reported that GPI-anchored folate receptors are clustered in microdomains of around 70 nm in size containing approximately 50 folate receptors (Mayor and Varma, 1998; Sabharanjak et al., 2002; Friedrichson and Kurzchalia, 1998).

Several drug carriers targeting the folate receptor have been studied. However, none of the studies published have accounted for the spatial distribution of receptors in designing optimal drug carriers. For instance, one would envision that folate-bearing

carriers that target cancer cells over-expressing folate receptors should be designed such that sufficient numbers of carriers bind on the cell surface to allow increased numbers of carriers to be internalized. Thus in the case of C6 and KB cell lines which both express folate receptors, one could hypothesize that an optimal design of the targeted liposomes would be such that each liposome binds to a single cluster. Therefore, depending on the density and distribution of receptors, one could design the targeted carrier to have an optimum tether length and an optimum number of ligands to allow the carrier to form sufficient bonds with receptors on the surface to prevent detachment and thus gain entry into the cell.

2.3.3 Folate conjugates are not rapidly degraded following internalization

Most of the ligands delivered into cells by endocytic pathways are internalized for the purpose of destruction. That is hormones that have already transduced their signals, as well as foreign particles that could be harmful to a cell if allowed to remain are endocytosed and rapidly trafficked to lysosomes where they are digested. In contrast, folic acid is internalized not for destruction, but rather for consumption. Not surprisingly, folate conjugates have been observed to remain stable and functional for hours following uptake by cancer cells (Leamon and Low, 1991). Thus, proteins and nucleic acids remain undigested, enzymes retain their activities, and liposomes resist disruption (unless engineered to disintegrate at endosomal pH) following uptake by FBP. Where sensitivity to hydrolytic enzymes such as nucleases, proteases, glycosidases and lipases is a concern, vitamin-mediated delivery may constitute a more protected pathway for intracellular delivery (Leamon and Low, 1991; Lee and Low, 1994; Wang et al., 1995).

2.3.4 Tumor cells overexpress folate receptors

The folate receptor as a tumor marker and simultaneously suggested that the folate-mediated delivery technology that we had been studying in cultured cells might be useful to target agents to cancer cells *in vivo*. Those cancers that were found to most prominently overexpress the α-isoform of the folate receptor included carcinoma's of the ovary, kidney, uterus, testis, brain, colon, and adeno carcinomas of the lung

(Campbell et al., 1991; Holm et al., 1994; Rettig et al., 1988; Ross et al., 1994; Weitman et al., 1992).

2.3.5 Folate-conjugated liposomes can be targeted to folate receptor-bearing cells

Liposomes are attractive as drug carriers because of their large cargo capacity and their compatibility. However, liposomes can also suffer from short blood circulation times and low tissue specificity. Lee and Low attempted to solve both of these problems by using stable, naturally occurring membrane lipids and linking of these lipids to folic acid via a polyethylene glycol (PEG) bridge. The resulting liposomes are both stable in serum and tumor-specific. Folate-PEG-liposomes display no tendency to fuse with or otherwise enter FBP-negative cells. However, the same liposomes are avidly internalized by receptor-bearing tumor cells, accumulating 5 to 10 liposomes/ cell within 1 h incubation. In fact, because each liposome is derivatized with several hundred molecules of folic acid, multiple tethers to each cancer cell are possible, generating an essentially irreversible binding interaction. In contrast, liposomes similarly derivatized with PEG alone (no folate) showed no tendency to enter the same cancer cells (Lee and Low, 1994; Wang and Low, 1998).

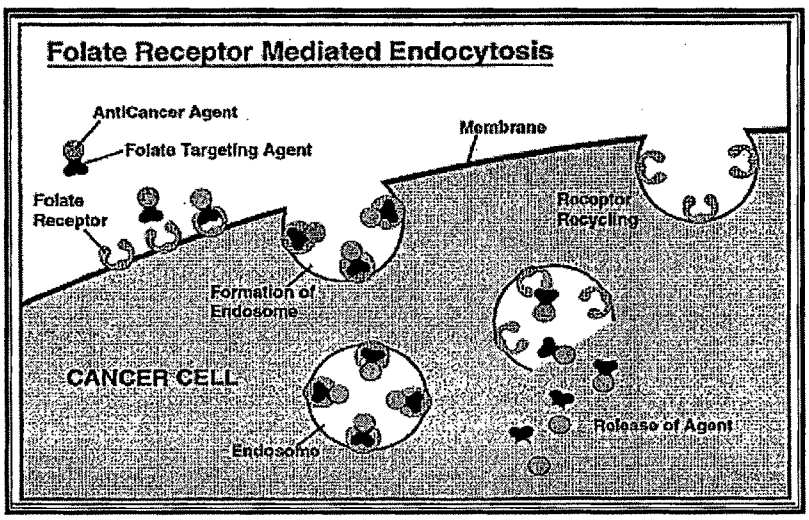

Figure 2.4 Diagrammatic representation of the folate receptor mediated endocytosis pathway. Covalent conjugates of delivery system linked to folic acid via the Folate's γ-carboxyl group bind to the folate receptor with equal affinity to free folic acid (K_D~50pM). Following endocytosis and vesicular trafficking, much of the material is released into the cell cytoplasm. The unligated folate receptor may then recycle to the cell surface. (Wang and Low, 1998)

2.3.6 Challenges for site-specific delivery

To successfully access target tissues, liposomes must be able to overcome several factors limiting their biodistribution and uptake. These limitations include short circulation half-lives, slow extravasation into target tissues and penetration into sites of action. Liposomes can enhance circulation half-life by decreasing glomerular filtration rate and protecting the active agent from metabolism and degradation while in the circulation. The choice of suitable lipid components can reduce MPS uptake while selection of the correct particle size can increase extravasation. Adhesion at the desired site of action (either within the tissue or at the surface of the targeted cells) can provide high local concentrations of active agent for uptake into target cells as shown in Fig. 2.5.

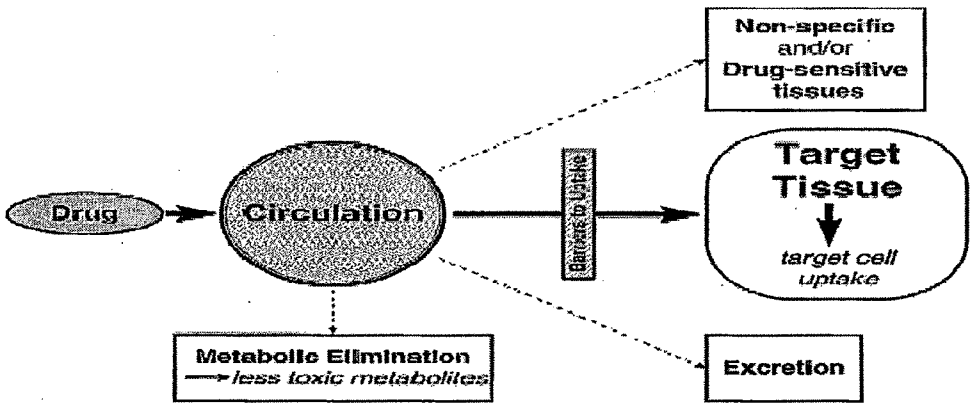

Figure 2.5 Challenges for site-specific liposomal drug delivery systems (Eric Forssena and Michael Willisb, 1998)

2.3.7 Uptake and interaction between liposomes and cells

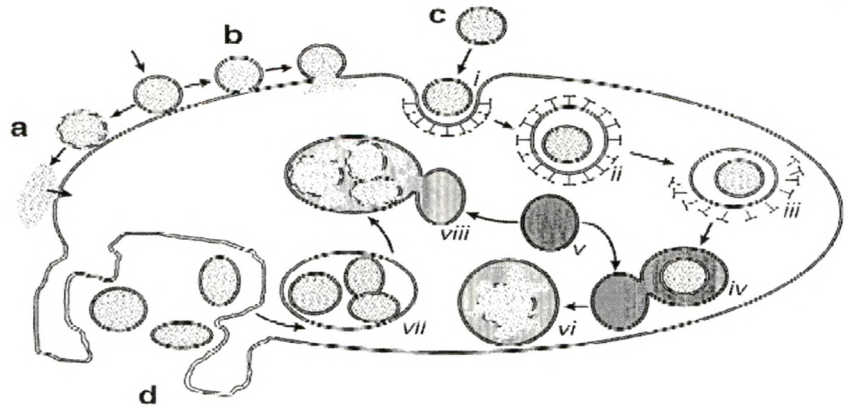

Figure 2.6 Principal modes of uptake and interaction between liposomes and cells. (a) Following surface absorption, liposomes may remain bound to the outer cell membrane where they may breakdown, releasing their entrapped contents to be absorbed as free drug. (b) Alternatively, surface absorbed liposomes may fuse with cells, delivering their contents directly to the cytoplasm. Active liposome internalization by cells can involve either receptor-mediated endocytosis or phagocytosis. (c) In receptor-mediated endocytosis, smaller particles (less than 150 nm diameter) bind to cell surface receptors (i) and are taken up into clathrin-coated pits, forming coated vesicles (ii). Following internalization, the clathrin coat is removed (iii). This is followed by fusion (iv) with lysosomes (v), breakdown of the lipids and release of entrapped contents (vi). (d) Larger particles (greater than 150 nm diameter) are taken up principally by phagocytosis (vii). Phagocytosis is usually limited to specific cells such as macrophages but can be induced in many other cell types with the appropriate ligand (Eric Forssena and Michael Willisb, 1998)

2.3.8 Summary

High tumor selectivity has been achieved both *in vitro* and *in vivo* experiments with folate-targeted antineoplastic drugs; compared to the more commonly exploited tumor-specific monoclonal antibodies, the vitamin, folic acid has many advantages as a tumor targeting agent. These include its low cost, high chemical and biological stability, compatibility with organic solvents, non-immunogenicity, stronger affinity for its receptor, and small size for fast tumor extravasation and thorough intercellular infiltration. Folate-mediated targeting systems should consequently have significant clinical potential, since overexpression of folate receptors has been found in a large fraction of human cancers but rarely in normal tissues, allowing high tumor specificity in the diagnosis and treatment of cancer.

2.4 Cervical Cancer

2.4.1 Introduction

The most important risk factor for cervical cancer, and abnormal pap smears, is infection with human papilloma virus (HPV). HPVs are a group or more than 100 types of viruses transmitted by skin to skin contact. A subset of 15 'high-risk' HPVs are associated with the occurrence of cervical cancer and pre-cancers. Most people clear their HPV infection, however chronic persistent infection with certain high-risk HPV infection is the strongest risk factor for developing cervical cancer. The United States Food and Drug Administration (FDA) approved the drug, Gardasil as a vaccine to prevent four types of the HPV virus.

Cervical cancer was once a major killer of women. The National Cancer Institute estimates the 80% decrease in incidence and mortality from cervical cancer can be attributed to the use of the Papanicolaou test or Pap test. Regular screening with a Pap test and other tools can detect abnormal cell changes in the cervix before they turn in to cancer. The Pap test allows gynecologists to find abnormal cells and destroy them before they become invasive cancer, women should have regular cervical cancer screenings to maintain their gynecological health.

2.4.2 Signs and Symptoms

Early-stage cervical cancer has no symptoms. When symptoms do develop, they may include painful intercourse, bleeding after intercourse, irregular bleeding between periods, heavy menstrual flow and abnormal vaginal discharge.

2.4.3 Screening and Diagnosis

An annual Pap test is the screening test for cancer of the cervix. If the Pap test detects abnormal cells, a biopsy of the cervix is done with the use of a colposcope (a magnifying glass) where the walls of the vagina and cervix are examined to determine if cancer is present.

2.4.4 Treatment

The choice of treatment depends upon precancerous or cancerous conditions and stage of the cancer. The precancerous condition called dysplasia or carcinoma *in situ* is treated with laser, LEEP or cryosurgery treatment. It is important for patients to be aware of their condition so they can be actively involved in the decision making process with their doctor. Cancer confined to the cervix is usually treated by radical surgery with removal of lymph nodes and advanced cancers are treated with radiation therapy. Chemotherapy is used along with radiation for patients who have cancer that has spread to other organs.

2.5 Lung Cancer

2.5.1 Introduction

Lung cancer is a disease of uncontrolled cell growth in tissues of the lung. This growth may lead to metastasis, which is the invasion of adjacent tissue and infiltration beyond the lungs. The vast majority of primary lung cancers are carcinomas of the lung derived from epithelial cells. Lung cancer, the most common cause of cancer-related death in men and women, is responsible for 1.3 million deaths worldwide annually as of 2004. The most common symptoms are shortness of breath, coughing (including coughing up blood) and weight loss (Minna and Schiller, 2008).

The main types of lung cancer are small cell lung carcinoma and non-small cell lung carcinoma. This distinction is important, because the treatment varies; non-small cell lung carcinoma (NSCLC) is sometimes treated with surgery, while small cell lung carcinoma (SCLC) usually responds better to chemotherapy and radiation. The most common cause of lung cancer is long-term exposure to tobacco smoke (Vaporciyan et al., 2000). The occurrence of lung cancer in nonsmokers, who account for as many as 15% of cases, is often attributed to a combination of genetic factors, radon gas, asbestos and air pollution, including second hand smoke.

Lung cancer may be seen on chest radiograph and computed tomography (CT scan). The diagnosis is confirmed with a biopsy. This is usually performed by bronchoscopy or CT-guided biopsy. Treatment and prognosis depend upon the histological type of cancer, the stage (degree of spread) and the patient's performance status. Possible treatments include surgery, chemotherapy and radiotherapy. Depending on the stage and treatment the five-year survival rate is 14% (Gorlova et al., 2007; Catelinois et al., 2006; Coyle et al., 2006).

2.5.2 Non-Small Cell Lung Carcinoma (NSCLC)

The non-small cell lung carcinomas are grouped together because their prognosis and management are similar. There are three main sub-types: squamous cell lung carcinoma, adenocarcinoma and large cell lung carcinoma. Accounting for 25% of lung cancers squamous cell lung carcinoma usually starts near a central bronchus. A hollow cavity and associated necrosis are commonly found at the center of the tumor.

Well-differentiated squamous cell lung cancers often grow more slowly than other cancer types (Travi, 2002; Vaporciyan et al., 2000). Adenocarcinoma accounts for 40% of lung cancers (Travi, 2002). It usually originates in peripheral lung tissue. Most cases of adenocarcinoma are associated with smoking; however, among people who have never smoked ("never-smokers"), adenocarcinoma is the most common form of lung cancer (Subramanian and Govindan, 2007). A subtype of adenocarcinoma the bronchioloalveolar carcinoma is more common in female; never-smokers may have different responses to treatment (Raz et al., 2006).

2.5.3 Small Cell Lung Carcinoma (SCLC)

Small cell lung carcinoma is less common. Also called oat cell cancer, it tends to arise in the larger airways (primary and secondary bronchi) and grows rapidly becoming quite large (Collins et al., 2007). The small cells contains dense neurosecretory granules (vesicles containing neuroendocrine hormones) which give this an endocrine/paraneoplastic syndrome association (Rosti et al., 2006). While initially more sensitive to chemotherapy, it ultimately carries a worse prognosis and is often metastatic at presentation. Small cell lung cancers are divided into limited stage and extensive stage disease. This type of lung cancer is strongly associated with smoking (Barbone et al., 1997).

2.5.4 Staging

Lung cancer staging is an assessment of the degree of spread of the cancer from its original source. It is an important factor affecting the prognosis and potential treatment of lung cancer. Non-small cell lung carcinoma is staged from IA ("one A"; best prognosis) to IV ("four"; worst prognosis). Small cell lung carcinoma is classified as limited stage if it is confined to one half of the chest and within the scope of a single radiotherapy field; otherwise it is extensive stage (Collins et al., 2007).

2.5.5 Symptoms (Hamilton et al., 2005)

- ✓ Dyspnea (shortness of breath)
- ✓ Hemoptysis (coughing up blood)
- ✓ Chronic coughing or change in regular coughing pattern

- ✓ Wheezing
- ✓ Chest Pain or pain in the abdomen
- ✓ Cachexia (weight loss), fatigue, and loss of appetite
- ✓ Dysphonia (hoarse voice)
- ✓ Clubbing of the fingernails (uncommon)
- ✓ Dysphagia (difficulty swallowing).

2.5.6 Causes

The main causes of any cancer include carcinogens (such as those in tobacco smoke), ionizing radiation and viral infection. This exposure causes cumulative changes to the DNA in the tissue lining the bronchi of the lungs (the bronchial epithelium). As more tissue becomes damaged, eventually a cancer develops (Vaporciyan et al., 2000).

- ✓ Smoking, particularly of cigarettes is by far the main contributor to lung cancer. Cigarette smoke contains over 60 known carcinogens, including radioisotopes from the radon decay sequence, nitrosamine and benzopyrene. Additionally nicotine appears to depress the immune response to malignant growths in exposed tissue. Across the developed world almost 90% of lung cancer deaths are caused by smoking. In the United States smoking is estimated to account for 87% of lung cancer cases (90% in men and 85% in women). Among male smokers the lifetime risk of developing lung cancer is 17.2%; among female smokers the risk is 11.6%. This risk is significantly lower in nonsmokers: 1.3% in men and 1.4% in women (Hecht, 2003; Sopori, 2002; Peto et al., 2006; Samet, 2006).

- ✓ Radon is a colorless and odorless gas generated by the breakdown of radioactive radium, which in turn is the decay product of uranium found in the Earth's crust. The radiation decay products ionize genetic material causing mutations that sometimes turn cancerous. Radon exposure is the second major cause of lung cancer after smoking (Catelinois et al., 2006).

- ✓ Asbestos can cause a variety of lung diseases including lung cancer. There is a synergistic effect between tobacco smoking and asbestos in the formation of lung cancer (O'Reilly et al., 2007).

- ✓ Viruses are known to cause lung cancer in animals and recent evidence suggests similar potential in humans. Implicated viruses include human papilloma virus, JC virus, simian virus 40 (SV40), BK virus and cytomegalo virus. These viruses may affect the cell cycle and inhibit apoptosis allowing uncontrolled cell division (Leroux et al., 2007; Palmarini and Fan. 2001).

2.5.7 Treatment

Treatment for lung cancer depends on the cancer's specific cell type, how far it has spread and the patient's performance status. Common treatments include surgery, chemotherapy and radiation therapy (Clegg et al., 2002).

2.5.8 Chemotherapy

Small cell lung carcinoma is treated primarily with chemotherapy and radiation, as surgery has no demonstrable influence on survival. Primary chemotherapy is also given in metastatic non-small cell lung carcinoma. The combination regimen depends on the tumor type. Non-small cell lung carcinoma is often treated with cisplatin or carboplatin, in combination with gemcitabine, paclitaxel, docetaxel, etoposide or vinorelbine. In small cell lung carcinoma, cisplatin and etoposide are most commonly used. Combinations with carboplatin, gemcitabine, paclitaxel, vinorelbine, topotecan, and irinotecan are also used in extensive-stage small-cell lung cancer, celecoxib may safely be combined with etoposide this combination showed improve outcomes (Clegg et al., 2002; Murray and Turrisi., 2006; Azim and Ganti., 2007; MacCallum and Gillenwater, 2006).

Reference

1. Azim HA, Ganti AK. Treatment options for relapsed small-cell lung cancer. Anticancer drugs 2007;18(3):255-261.
2. Barbone F, Bovenzi M, Cavallieri F, Stanta G. Cigarette smoking and histologic type of lung cancer in men. Chest 1997;112(6):1474-1479.
3. Brandl M. Liposomes as drug carriers: a technological approach. Biotechnol Annu Rev 2001;7:59-85.
4. Campbell IG, Jones TA, Foulkes WD, Trowsdale J. Folate-binding protein is a marker for ovarian cancer. Cancer Res. 1991;51:5329-5338.
5. Catelinois O, Rogel A, Laurier D, et al. Lung cancer attributable to indoor radon exposure in france: impact of the risk models and uncertainty analysis. Environ. Health Perspect. 2006;114(9):1361-1366.
6. Clegg A, Scott DA, Hewitson P et al. Clinical and cost effectiveness of paclitaxel, docetaxel, gemcitabine, and vinorelbine in non-small cell lung cancer: a systematic review. Thorax 2002;57(1):20-28.
7. Collins LG, Haines C, Perkel R, Enck RE. Lung cancer: diagnosis and management. American Family Physician 2007;75(1):56-63.
8. Coyle YM, Minahjuddin AT, Hynan LS, Minna JD. "An ecological study of the association of metal air pollutants with lung cancer incidence in Texas". Journal of Thoracic Oncology 2006;1(7):654-661.
9. D.A. Eavarone, X. Yu, R.V. Bellamkonda. Targeted drug delivery to C6 glioma by transferrin-coupled liposomes, J. Biomed. Mater. Res. 2000;51:10-14.
10. Eric Forssena, Michael Willisb. Ligand-targeted liposomes. Advanced Drug Delivery Reviews 1998;29:249-271.
11. Friedrichson T, Kurzchalia TV. Microdomains of GPIanchored proteins in living cells revealed by crosslinking. Nature 1998;394:802-805.
12. Gabizon A, Horowitz AT, Goren D, et al. Targeting folate receptor with folate linked to extremities of poly(ethylene glycol)-grafted liposomes: in vitro studies, Bioconjug. Chem. 1999;10:289-298.
13. Gemcitabine Hydrochloride. [Online]. [cited 2006 May 6]; Available from: URL:http://www.cancer.gov/Templates/drugdictionary.aspx?CdrID=41213

14. Gorlova OY, Weng SF, Zhang Y et al. Aggregation of cancer among relatives of never-smoking lung cancer patients. International Journal of Cancer 2007;121(1):111-118.
15. Hamilton W, Peters TJ, Round A, Sharp D. What are the clinical features of lung cancer before the diagnosis is made? A population based case-control study. Thorax 2005; 60(12):1059-1065.
16. Hecht S. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews. Cancer 2003;3(10):733-744.
17. Holm J, Hansen SI, Hoier-Madsen M, Sondergaard K, Bzorek M. Folate receptor of human mammary adenocarci noma, APMIS. 1994;102:413-419.
18. Jacobson K, Dietrich C. Looking at lipid rafts? Trends Cell Biol. 1999;9:87-91.
19. Johnson SA. Clinical pharmacokinetics of nucleoside analogues: focus on haematological malignancies. Clin Pharmacokinet 2000;39:5-26.
20. Kurihara A, Deguchi Y, Pardridge WM. Epidermal growth factor radiopharmaceuticals: ^{111}In chelation, conjugation to a blood–brain barrier delivery vector via a biotin–polyethylene linker, pharmacokinetics, and *in vivo* imaging of experimental brain tumors, Bioconjug. Chem. 1999;10:502-511.
21. Lappi DA. Tumor targeting through fibroblast growth factor receptors. Semin Cancer Biol. 1995;6:279-288.
22. Leamon CP, Low PS. Delivery of macromolecules into living cells: a method that exploits folate receptor endocytosis. Proc. Natl. Acad. Sci. USA 1991;88:5572-5576.
23. Lee RJ, Low PS. Delivery of liposomes into cultured KB cells via folate receptor-mediated endocytosis, J. Biol. Chem. 1994;269:3198-3204.
24. Lee RJ, Low PS. Delivery of liposomes into cultured KB cells via folate receptor-mediated endocytosis. J. Biol. Chem. 1994;269:3198-3204.
25. Lee RJ, Low PS. Folate-mediated tumor cell targeting of liposome-entrapped doxorubicin in vitro. Biochim. Biophys. Acta 1995;1233:134-144.
26. Leroux C, Girard N, Cottin V et al. Jaagsiekte Sheep Retrovirus (JSRV): from virus to lung cancer in sheep. Veterinary Research 2007;38(2):211-228.

27. MacCallum C, Gillenwater HH. Second-line treatment of small-cell lung cancer. Current Oncology Reports 2006;8(4):258-264.
28. Massing U, Fuxius S. Liposomal formulations of anticancer drugs: selectivity and effectiveness. Drug Resist Update 2000;3:171-177.
29. Mayor S, Varma R. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature 1998;394:798-801.
30. Minna JD, Schiller JH. Harrison's Principles of Internal Medicine (17th ed.). McGraw-Hill. 2008;551-562.
31. Moog R, Burger AM, Brandl M, et al. Change inpharmacokinetic and pharmacodynamic behaviour of gemcitabine in human tumorxenografts upon entrapment in vesicular phospholipid gels. Cancer Chemother Pharmacol. 2002;49:356-366.
32. Murray N, Turrisi AT. A review of first-line treatment for small-cell lung cancer. Journal of Thoracic Oncology 2006;1(3):270-278.
33. New R. Introduction. In: New R, editor. Liposomes a practical approach. 1st ed: Oxford University Press; 1990;1-32.
34. Noble S, Goa KL. Gemcitabine. A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. Drugs 1997;54(3):447-472.
35. O'Reilly KM, Mclaughlin AM, Beckett WS, Sime PJ. Asbestos-related lung disease. American Family Physician 2006;75(5):683-688.
36. Palmarini M, Fan H. Retrovirus-induced ovine pulmonary adenocarcinoma, an animal model for lung cancer. Journal of the National Cancer Institute 2001;93(21):1603-1614.
37. Peto R, Lopez AD, Boreham J et al. Mortality from smoking in developed countries 1950-2000: Indirect estimates from National Vital Statistics. Oxford University Press. 2006.
38. Raz DJ, He B, Rosell R, Jablons DM. Bronchioloalveolar carcinoma: a review. Clinical Lung Cancer 2006;7(5):313-322.

39. Rettig W, Garin-Chesa P, Beresford H, et al. Cell-surface glycoproteins of human sarcomas: differential expression in normal and malignant tissues and cultured cells, Proc. Natl. Acad. Sci. USA 1988;85:3110-3114.
40. Ross JF, Chaudhuri PK, RatnamM. Differential regulation of folate receptor isoforms in normal and malignant tissues *in vivo* and in established cell lines - Physiologic and clinical implications, Cancer 1994;73:2432-2443.
41. Rosti G, Bevilacqua G, Bidoli P et al. Small cell lung cancer. Annals of Oncology 2006; 17(Suppl. 2):5-10.
42. Sabharanjak S, Sharma P, Parton RG, Mayor S. GPIanchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. Dev. Cell Biol. 2002;2:411-423.
43. Samet JM, Wiggins CL, Humble CG, Pathak DR. Cigarette smoking and lung cancer in New Mexico. American Review of Respiratory Disease 1998;137(5):1110-1113.
44. Shamseddine AI, et al. Comparative pharmacokinetics and metabolic pathway of gemcitabine during intravenous and intra-arterial delivery in unresectable pancreatic cancer patients. Clin Pharmacokinetis 2005;44:957-67.
45. Sheets ED, Lee GM, Simson R, Jacobson K. Transient confinement of a glycosyl phosphatidylinositol-anchored protein in the plasma membrane, Biochemistry 1997; 36:12449-12458.
46. Sopori M. Effects of cigarette smoke on the immune system. Nature Reviews. Immunology 2002;2(5):372-377.
47. Subramanian J, Govindan R. Lung cancer in never smokers: a review. Journal of Clinical Oncology 2007;25(5):561-570.
48. Travis WD. Pathology of lung cancer. Clin Chest Med. 2002;23:65-81
49. Vaporciyan AA, Nesbitt JC, Lee JS, et al. Cancer of the Lung. In: Bast RC, Kufe DW, Cancer Medicine, 5th ed. Ontario: BC Decker, Inc. 2000:1227-1292.
50. Villeneuve PJ, Mao Y. Lifetime probability of developing lung cancer, by smoking status. Canadian Journal of Public Health 1994;85(6):385-388.

51. Wang S, Lee RJ, Cauchon G, Gorenstein DG, Low PS. Delivery of antisense oligonucleotides against the human epidermal growth factor receptor into cultured KB cells with liposomes conjugated to folate via polyethyleneglycol. Proc. Natl. Acad. Sci. USA 1995;92:3318-3322.
52. Wang S, Lee RJ, Cauchon G, Gorenstein DG, Low PS. Delivery of antisense oligodeoxy -ribonucleotides against the human epidermal growth factor receptor. Proc. Natl. Acad. Sci. U SA. 1995;92:3318-3322.
53. Wang S, Low PS. Folate-mediated targeting of anti neoplastic drugs, imaging agents, and nucleic acids to cancer cells. J. Control. Release 1998;53:39-48.
54. Weitman SD, Lark RH, Coney LR, et al. Distribution of the folate receptor (GP38) in normal and malignant cell lines and tissues. Cancer Res. 1992;52:3396-3401.