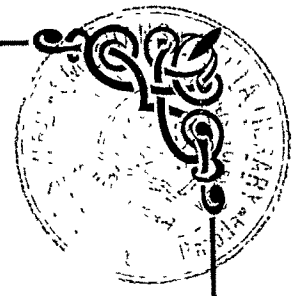

Chapter 1

Introduction

1.1 INTRODUCTION

Cancer, perhaps the most feared of all diseases, has become the second leading cause of death for the past 50 years. Worldwide, every year more than 10 million people are diagnosed with cancer, and more than 6 million die from cancer. By 2020, WHO estimates that there will be some 20 million new cancer patients in the world each year. More than 70% of these patients will be in developing countries. The term cancer covers more than 100 different diseases, which arise principally as a consequence of exposure to cancer causing agents (carcinogens). Researchers are engaged consistently in developing a possible cure.

Due to the high mortality rate, the chemotherapy of cancer has been under vigorous investigation and important gains have been made in the treatment of various neoplasms include testicular germ cell carcinoma, chorio carcinoma, Burkitt's lymphoma, Hodgkin's disease and several childhood cancers. However progress in treating the three solid malignancies of breast, lung and colon is less impressive. The limiting factors of the effectiveness of cancer chemotherapy are,

1. The failure to differentiate the tumor cells from the normal cells results in causing host tissue toxicity.
2. The cellular and sub cellular differences between tumor cells and their normal counterparts appear to be quantitative rather than qualitative.
3. Carcinogenicity and teratogenecity of the anticancer agents.
4. Enormous side effects of the drugs used for cancer.
5. Development of Multidrug resistance.

Towards this objective, considerable pharmaceutical and pharmacological research has been devoted to develop variety of drug carrier systems. The medical challenge for directing the administered drug preferentially to tumor cells can be achieved by the use of appropriate vehicle system. One such promising delivery system for the cancer therapy is the liposome, which was brought to the attention of the scientific world by Bangham in 1965.

Liposome is one of the attractive systems for targeted drug delivery due to its composition from natural biological lipids and structural resemblance to cell membranes which suggests metabolic compatibility. Liposomes are phospholipid vesicles or microscopic particles composed of a lipid bilayer membrane. Their nature of encapsulating an aqueous phase within the lipid bilayer suggests the possibility of both hydrophilic and lipophilic drugs to be encapsulated and delivered easily. The major advantages of using liposomes as target drug delivery systems are,

1. better therapeutic index and enhanced cellular uptake.
2. biodegradability.
3. low toxicity.
4. low immunogenicity.
5. ability to trap drugs without the necessity of covalent bonding.
6. potential for high drug load capacity.
7. ease of preparation in a variety of forms.
8. ability for sustained and controlled release of drugs.

However, liposomes have been limited by a number of factors such as,

1. relatively fast size dependant clearance.
2. tendancy to localize in the tissues of the mononuclear phagocyte system (MPS), particularly of the liver and spleen.
3. limited tendency to distribute to non-MPS tissues.
4. stability.
5. scale-up difficulties.

Thus conventional liposomes along with their associated drugs often eliminated from the circulation by cells of the MPS before effective delivery of drugs to extravascular and some intravascular target sites can be achieved. Uptake by the MPS cells generally leads to irreversible sequestering of the unencapsulated drugs thereby eliminating any beneficial effects as well as posing potential risk of toxicity to these cells.

One of the first major advances in prolonged blood residence was made possible by the observations that small (≤ 100nm diameter), electrically neutral, rigid liposomes can exhibit prolonged circulation at moderately high doses probably by saturation of the uptake mechanism. Another important landmark in the process of reduction of the MPS uptake of liposomes was the inclusion of special biomolecules in the liposomes like monoganglioside (GM_1), hydrogenated phosphatidyl inositol and sulphatides, which, when included in small, rigid and neutral liposomes led to significant MPS avoidance. This was followed by findings that a hydrophilic polymer-lipid conjugate (polyethylene glycol-phosphatidyl ethanolamine or PEG-PE) could provide similar or even better protection from uptake with little dependence on liposome composition. PEG-PE imparts special surface properties to liposomes due to which they have several advantages for drug delivery such as dose independent pharmacokinetics, the ability to independently adjust the liposomal physicochemical properties (drug loading and leakage); biological properties (blood circulation and tissue distribution); ease of preparation and use without modification of the many well known liposomal methodologies. These liposomes, also known as sterically stabilized liposomes are also amenable to pharmaceutical product regulatory requirements.

At present studies are going on for investigating the potential if other polymer-lipid conjugates for their ability to bring about steric stabilization of liposomes. The focus is also on the probable mechanisms of this kind of stabilization. Such studies will greatly enhance the applicability of these findings to routine therapy.

The aim of the present study was to develop delivery systems containing anticancer drugs with prolonged circulation time, slow the systemic delivery and increase the accumulation in areas of interest i.e. tumors. It was conjectured that the intercalation of anticancer drugs in liposomes would protect them from the kinetic processes in the body. The long circulation of sterically stabilized liposomes in the blood due to their avoiding uptake by the MPS would mean increased accumulation and retention of drugs to the tumor tissue due to its leaky vasculature compared with those in normal

tissue and the phenomenon now known as the EPR (enhanced permeability and retention) effect of macromolecules and lipidic particles in solid tumor.

Sustained and targeted delivery not only would minimize interactions of these drugs with other tissues of the body thereby reducing side effects commonly associated with this type of therapy but also would lead to significant dose reduction. Thus overall increase in therapeutic efficacy would be achieved.

The anticancer drugs selected for the studies are as follows:

Methotrexate (MTX), an antimetabolite of folic acid analog type is one of the most widely used anticancer agents. Methotrexate is indicated for treatment of breast carcinoma, head and neck cancer, lung, cervical, ovarian, bladder, colorectal, esophageal, gastric, pancreatic and penile carcinoma. Tamoxifen citrate (TMX) is the most commonly used drug for breast cancer and ductal carcinoma. In addition, it is currently being considered for widespread use in healthy women for breast cancer prevention. Cytarabine (CYT) is one of the most important antimetabolites used in the therapy of acute myelocytic leukemia. It is the single most effective anticancer agent for the induction of remission in this disease. Cytarabine is also used in acute granulocytic and acute lymphatic leukemia. These anti cancer drugs are known to be potent myelosuppresants and the therapeutic index of these anticancer drugs are very narrow which demands the need for targeted delivery systems for delivering the drugs to the tumor site.

1.2 PROPOSED PLAN OF WORK

The proposed plan of work is as follows:

1. Preparation of conventional liposomes of MTX, TMX and CYT and optimization of the process and formulation variables.
2. Synthesis and characterization of methoxy polyethylene glycol derivatives using cyanuric chloride and carbonyl diimidazole as coupling agents.
3. Preparation of sterically stabilized liposomes by incorporating the prepared polymers (mPEG5000-CC-PE, mPEG2000-CC-PE and

mPEG5000-IC-PE) into the formulations of the conventional liposomes of MTX, TMX and CYT prepared above, and optimization of the process.

4. Characterization of the conventional and sterically stabilized liposomes of MTX, TMX and CYT by methods useful for ascertaining the well documented liposomal parameters (size, shape, lamellarity, entrapment, entrapped volume etc.) and the extent of steric stabilization.
5. *In vitro* drug release studies, using an appropriate method, for evaluation of the sustained release of MTX, TMX and CYT from conventional and sterically stabilized liposomes.
6. *In vitro* cytotoxicity studies of the conventional and sterically stabilized liposomes of MTX, TMX and CYT using cancer cell lines by MTT assay.
7. Optimization of radiolabeling of the free drugs (MTX, TMX and CYT) and their prepared liposomal formulations using Technetium (^{99m}Tc).
8. *In vivo* biodistribution studies of the prepared liposomes for the evaluation of sustained and targeted delivery using suitable animal models.
9. Comparison of myelosuppresive action of the free drugs (MTX, TMX and CYT) and their prepared liposome formulations.