Introduction

In 1965, Bengham formulated vesicles from lipids and named as Liposome. That's base of new drug delivery for the cancer and many other diseases. The first ever made vesicles are Multi Lamellar Vesicles [MLV] made up from egg lecithin.

What we know is a drop. What we don't know is an ocean. (Isaac Newton)
1.1 INTRODUCTION

National Cancer Institute announced its strategic plan 2007- To eliminate suffering and death due to cancer. The scientific communities of the National Cancer Institute [NCI] have announced that they eliminate suffering from cancer by 2015. In the plan they have focused on understanding of cause and mechanism of cancer for effective treatment. Cancer caused 5 millions deaths in 2006 only in USA [Cancer facts and figure-2006]. Last decade was the most important for the cancer treatment, as research has been transferred to molecular level. Therefore now a closer look has been taken for the processes which govern the progress of cancer development. The cancer is like evil which leads to death and this is happened due to uncontrolled cells growth. Cancer is not fatal unless it becomes malignant, once cancerous cells migrate from one site to other and becomes evil which is understood as metastasize and process of spreading of cancer cells known as Metastasis. In the malignancy the cells established colony at distant organ and grow as new cancer foci. The process of metastasis is very complex and before the cells to become malignant it passes through several steps like invasion, angiogenesis, migration and attachment. This brainteaser existed in literature from the time of Mesopotamian and/or Babylonian [Wessig, 2000].

To metastasize, cancer cells successfully complete a series of sequential and highly selective steps to rise to secondary cancer and whose outcome is determined by the interactions of cancer cells with homeostatic host mechanisms. Likely cancer cells detached from a primary tumor, invade and/or circulate via the bloodstream, anchor down in a near and/or distant tissue or organ, and begin a new colony that might compromise the function of that organ. Due to strong immune system response of body one of million cells are able to establish as secondary cancer foci. Initially cancerous cells invade in near vicinity affects adjacent organ which further increases in size due to fast growth. Increase in the cancer size requires more nutrients to fulfil metabolic needs; hence there is a formation of new capillaries and the process involved is called Angiogenesis. Detachment of cells from the primary tumor comes about by proteolytic enzymes and cells enter into the circulatory system- known as Intravasation. Eventually a cancer cell(s) may lodge in a capillary, adhere to and penetrate the capillary wall and migrate into the tissue and grow as new entity-secondary cancer. Invasion and metastasis kill hosts through two processes: local
invasion and distant organ injury. The candidate includes telomerase gene (hTERT), Ras, p53, pRb and PP2A proteins, MMPs, adhesion molecule mainly glycol proteins-cadherins, integrin, fibronectin, laminin, vitronectin plays major role in the process of metastasis.

The process of metastasis can be divided in the three basic steps: departure of cell(s) from primary tumors, penetration in to blood or lymph vessels and establishment of new colonies. Epithelial cells are the most common originator of cancer; they compromise the outer surface of the skin and the lining of the gut, lungs and several other organs [Ruoslahti, 1996]. In order to leave the epithelium the metastatic cells must overcome the adhesion interaction, which keeps the trillions of the normal cells fixed in their proper locations. There are two primary mechanisms that must metastatic cells overcome: Cell-cell adhesion (a critical early step in metastasis) and cell-Extra Cellular Matrix (ECM) adhesion [Chambers et al., 2002].

Interaction of cells with macromolecular components of ECM plays crucial role in regulation of cell morphology, growth, differentiation, adhesion, migration as well as spreading of cells. The phenomenological observation on cell-matrix interactions was suspected since long time by many of the researcher. But last two-decade is the time of progress in understanding of such complex process and biological basis of cell-matrix interactions. The curtain rise when the scientist found the role of glycoprotein, present in the ECM, with the cell adhesion, migration, and differentiation and also spreading of cell. Glycoprotein like integrin, fibronectin, laminin and vitronectin proved to have their role in the process of metastasis [Hart and Saini, 1992].

Current cancer therapy usually involves intrusive processes including application of catheters to allow chemotherapy, initial chemotherapy to shrink any cancer present, surgery to then remove the tumor(s) if possible, followed by more chemotherapy and radiation. The purpose of the chemotherapy and radiation is to kill the tumor cells. However, these cells are more susceptible to the actions of chemotherapeutic agents and methods because of their growth at a much faster rate than healthy cells, at least in adults. If the cancer is not treated in the early staged it may metastasize and become more difficult to treat because of the poorly understood process of metastasis and therefore no specific modalities would be adopted. In the large number of patients at
the time of diagnosis, cancer must have already established and/or started their second
its second lap of process and in this race patient rarely wins. In view of failure to treat
order by surgery, radiation therapy and non-specific toxicity of most
chemotherapeutic agents against normal cells, development of targeted chemotherapy
is warranted.

Research efforts to improve chemotherapy over the past 25 years have led to an
improvement in patient survival but there is still a need for improvement. The
nanoparticulate drug delivery systems like liposomes, nanoparticles etc., are proved to
be promising for the treatment of the cancer. As nanoparticles overcome Reticulo
Endothelial System (RES) therefore prolonged circulation in blood and again retained
in the tumor vasculature by Enhanced Permeability and Retention (EPR) effect [the
cancer have leaky capillaries in which the nanoparticles retained]. But such drug
carriers can not differentiate between the normal and malfunctioned cells and
therefore lack of specificity leads to toxicity. Current research areas include
development of carriers to allow alternative dosing routes, new therapeutic targets
such as blood vessels fueling tumor growth and targeted therapeutics that are more
specific in their activity. The era is dedicated to develop the better drug delivery with
effect targeting towards the tumor or metastasis. In the early efforts the researcher
targeted the tumor by attaching the antibody fragments on the surface of carriers like
liposomes [Rollrand et al., 1987]. The comparative results were obtained but still
there was lacking in specificity and at the same time the antibody produced
immunogenic response [Mishra and Jain, 2003]. In consecutive efforts receptor
mediated drug carriers were tried by coupling protein or carbohydrate moiety on the
surface [Annelies et al., 2002]. Despite the problems in handling of the protein and
carbohydrate molecules due to its higher molecular weight this carriers gain
somewhat popularity in research. In late 90’s, Iwomoto et al (1987) were represented
the YIGSR, a peptide sequence from laminin that have ability to prevent metastasis
when induced in mice with B16F10 melanoma cells. This peptide has intrinsic
property to inhibit the adhesion of the cancerous cells to the basal lamina and exposed
as antimetastatic agents. In preceding research the other anti adhesive peptides like
RGD, YIGSR, EILDV etc, were evaluated in metastatic tumor in vitro and in vivo. In
general these peptide sequences expressed via the integrin family and integrin
receptors are the key factor for the expression. In specific YIGSR, exerts their effect
by binding with 67 kD laminin receptor, RGD and EILDV specifically binds to integrin receptor and exerts their antiadhesive effect. Till date among all glycoproteins, integrin and fibronectin has been well studied; the reports reveals that tripeptide RGD (Arg-Gly-Asp), a short amino acid sequence posses antimetastatic activity [Hojo et al., 2001].

The work has been carried out in the last decade to study the role of another glycoprotein Laminin, in the process of metastasis. Laminin, a high-molecular-weight glycoprotein is a major component of basement membrane, which participates in several important biological activities including cellular attachment, differentiation, proliferation, and migration [Beck et al., 1990; Kleinman et al., 2001; Colognato and Yurchenco, 2000]. Interactions between cancer cells and laminin have been shown to play a critical role during tumor invasion and metastasis [Beck et al, 1990]. Laminin is made up of three chain α, β, γ, which form a cross like shape. It has been demonstrated that such activities are functionalized by some of the peptide sequences present within laminin. Most of the work was carried out on pentapeptide, present in β1 chain of laminin, which express its antiadhesive and/or antimetastatic activity by binding to high affinity, non-integrin laminin receptor with an apparent molecular weight of 67 kDa [Me'nard et al., 1997]. The expression of 67 kDa is more in tumor cell than the normal one. Along with these properties pentapeptide also inhibits the morphological differentiation of endothelial cell into capillary-like structures. Such biological properties make the pentapeptide a suitable ligand for tumor targeting. Other glycoproteins such as fibronectin have role in the process of metastasis and the pentapeptide sequence-EILDV has similar effects like YIGSR of laminin i.e. antimetastastic property.

However, these pentapeptides have some demerits like degradation by enzymes, have short half lives, faster renal clearance which are the common problems exists with other bioactive peptides[Lee, 1991]. The attempts were made to improve the stability of these peptides by attaching it to the water soluble polymers [Tsutsumi et al., 1995; Tsutsumi et al., 1997; Kaneda et al., 1998; Kawasaki et al., 1991]. In order to enhance the therapeutic potency of bioactive peptides, the development of an appropriate drug delivery system is required to improve in vivo stability. Strategies

INTRODUCTION

by binding with 67 kD laminin receptor, RGD and EILDV specifically binds to integrin receptor and exerts their antiadhesive effect. Till date among all glycoproteins, integrin and fibronectin has been well studied; the reports reveals that tripeptide RGD (Arg-Gly-Asp), a short amino acid sequence posses antimetastatic activity [Hojo et al., 2001].

The work has been carried out in the last decade to study the role of another glycoprotein Laminin, in the process of metastasis. Laminin, a high-molecular-weight glycoprotein is a major component of basement membrane, which participates in several important biological activities including cellular attachment, differentiation, proliferation, and migration [Beck et al., 1990; Kleinman et al., 2001; Colognato and Yurchenco, 2000]. Interactions between cancer cells and laminin have been shown to play a critical role during tumor invasion and metastasis [Beck et al, 1990]. Laminin is made up of three chain α, β, γ, which form a cross like shape. It has been demonstrated that such activities are functionalized by some of the peptide sequences present within laminin. Most of the work was carried out on pentapeptide, present in β1 chain of laminin, which express its antiadhesive and/or antimetastatic activity by binding to high affinity, non-integrin laminin receptor with an apparent molecular weight of 67 kDa [Me'nard et al., 1997]. The expression of 67 kDa is more in tumor cell than the normal one. Along with these properties pentapeptide also inhibits the morphological differentiation of endothelial cell into capillary-like structures. Such biological properties make the pentapeptide a suitable ligand for tumor targeting. Other glycoproteins such as fibronectin have role in the process of metastasis and the pentapeptide sequence-EILDV has similar effects like YIGSR of laminin i.e. antimetastastic property.

However, these pentapeptides have some demerits like degradation by enzymes, have short half lives, faster renal clearance which are the common problems exists with other bioactive peptides[Lee, 1991]. The attempts were made to improve the stability of these peptides by attaching it to the water soluble polymers [Tsutsumi et al., 1995; Tsutsumi et al., 1997; Kaneda et al., 1998; Kawasaki et al., 1991]. In order to enhance the therapeutic potency of bioactive peptides, the development of an appropriate drug delivery system is required to improve in vivo stability. Strategies
such as peptide-cyclization and D-amino acid substitution have been reported to enhance the therapeutic potency of antimitic peptides with improved physicochemical stability of the steric structure and improved biological stability by inhibiting enzymatic degradation [Kaneda et al, 1997; Kumagai et al, 1991].

Liposomes are the vesicles composed of a phospholipid bilayer in which pharmaceutical agents can be encapsulated. Resemblance of the membrane structure to cell membranes makes liposome's non-immunogenic and diversifies intake methods. Thus the liposome may serve as suitable candidate for attachment of the peptide as ligand to its surface. Numerous methods have been tried in the last two decades to increase the interaction between liposomes and the cells. In order to achieve the targeting of liposomes, which carry drugs or other bioactive molecules, is indeed necessary that the vesicles bind to their target cells with high selectivity and affinity. A fruitful approach is to attach various ligands to the surface of liposomes, which recognize by receptors over expressed on the target cells.

Another reason to conjugate a ligand to the surface of vesicles is the possibility of increasing their intracellular delivery; indeed in many cases the uptake of such targeted liposomes by cells is receptor-mediated (endocytosis and phagocytosis) i.e. process are much more efficient than the passive uptake of non-targeted liposome.

In the present study etoposide was used as an anticancer agent. Etoposide [VP 16] (ETO) is semi-synthetic derivative of epipodophyllotoxin is effectively used for the treatment of lung cancer, testicular cancer, lymphoma and several type of leukemia. It is one of the most active drugs against small cell lung cancer. Etoposide was synthesized from podophyllotoxin in 1963. Several phodophyllin components possess considerable antineoplastic activity but are very toxic for human use. Etoposide proved to be one of the most promising compounds. Podophyllin resin derivatives have been used as medicaments for over 250 years. In 1861 Bentley reported cytotoxic activity for podophyllin, which in a later report was useful as a topical solution in oil for treating condyloma acuminatum. Etoposide is a semi synthetic podophyllotoxin derived from the root of Podophyllum peltatum (the May apple or mandrake). It is known to cause single-strand breaks in DNA. Etoposide also causes DNA damage through inhibition of topoisomerase II and activation of oxidation-reduction reactions to produce derivatives that bind directly to DNA. Topoisomerase
II results in breakage and reunion reactions of DNA which are necessary for normal cellular function. Etoposide is cell cycle phase specific with predominant activity occurring in late S-phase and G₂.

The etoposide, used in combination with other antineoplastic agents, is effective in the treatment of tumors of the testis and lungs. It has been also used to treat other solid tumors including those of the brain, gastrointestinal tract, ovary, thymus and some childhood neoplasms; in lymphomas, acute leukemia and in the treatment of Kaposi's sarcoma associated with AIDS. The etoposide suffers from some unwanted side effects like decreased white blood cell count with increased risk of infection, decreased platelet count with increased risk of bleeding, mild nausea, mild vomiting, loss of appetite, changes in taste including metallic taste of foods.

Here the attempts were made to encapsulate etoposide within the liposomes to a maximum capacity and in subsequent steps the peptides [YIGSR/EILDV] were coupled to the distal end of the linkers [NGPE and DSPE-PEG-COOH], used to anchor the peptides to the carrier i.e. liposomes. These formulations might over come the problems related to the antibody and protein and carbohydrate molecules. As the antibody may create immunogenic problems and the high molecular structure of the protein renders its use as ligand.

1.2. AIM AND OBJECTIVES

To develop the drug delivery system that will target specific cells, release the encapsulated material from the liposomal delivery system and prevent the spread of the cancer.

- Preparation of liposome encapsulating etoposide (ETO)
- Characterization of prepared liposomes for particle size, entrapment efficiency, flocculation test, in vitro release and freeze drying [lyophilization]
- Attaching the peptide [YIGSR or EILDV] as ligand, on the surface of liposomes
- Evaluation of the parameters like particle size, entrapment efficiency, z-potential,
To determine the effect of cholesterol, pegylation, and freeze drying (cryoprotectant) on the particle size, entrapment efficiency, and z-potential.

- Evaluation of in vitro release and stability studies
- Evaluating the efficacy of the developed system on cell lines (in-vitro) includes cytotoxicity assay, colony formation assay, migration assay, adhesion assay, cell cycle analysis, and cellular uptake studies.
- Biodistribution study of the formulations
- Antimetastatic activity assay in mice (in-vivo)
REFERENCE


