A REVIEW ON: LIPOSOMAL PREPARATION OF TAMOXIFEN USED IN BREAST CANCER.

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Abstract: In this review article we discussed the types, preparation and evaluation of liposomal formulation of tamoxifen for the breast cancer. The breast cancer remains the most common cancer among women world wide prevailing disease world wide that requires, effective and rational therapy for this purpose the use of varies treatments modalities should be optimized. According to the risk benefits ratio of the therapeutic agents employed in the patients. Tamoxifen is an oral estrogen antagonist drug. Which is an adjust treatment of breast cancer when used in low doses. Liposomes are spherical vesicles consisting one or more phospholipid bilayer which are under extensive investigation as drug carrier for impairing the delivery of bioactive agent and many different compounds in biological. The Tamoxifen encapsulated in liposome by different methods like modified either Injection method (MEIM) & thin film Hydration method (TFHM) etc. The drug encapsulation in liposomes has provided an opportunity to improve therapeutic drug mainly through changes in biodistribution and targeting of drug to certain tissue. The role of liposomes as a drug delivery system is to provide drug in controlled manner.

Index Terms - Tamoxifen, Breast Cancer, Liposomes, Formulation of Tamoxifen Liposome, Evaluation of Liposomes.

I. INTRODUCTION

BREAST CANCER

The most common malignancy in among women worldwide is still breast cancer. Advances in the treatment of the disease have been made available by the rising incidence of breast cancer. Hence, local and systemic anti-cancer therapies increases patient survival outcomes, such as overall survival and survival without diseases. However, because diseases identification and therapy have advanced considerably over the years, quality of life is now considered a key outcome metric in clinical studies and survivorship studies in addition to on. [1]

The most common kind of cancer and the second biggest cause of death is breast cancer. This condition is the second largest cause of the cancer-related death and the top cancer of mortality in women between the ages of 45 and 55. Breast cancer kills almost one in eight women and is often treated with full tissue removal, chemotherapy, radiation, and hormone treatment. Breast cancer is tubes that carry the milk. Age, high hormone levels, race, economic situation, and iodine deficiency in diet are the man risk factors for cancer. Viruses contribute to one stage of the pathogenic process in the multi-stage diseases of breast cancer. [2]

With 14% of all AYA cancer diagnoses occurring in women between the ages of 25 and 39, breast cancer is the most common tumour among this group. Age raises the risk of breast cancer, however postmenopausal women have seen a dramatic decline in the incidence of the diseases, in part due to a lower utilization of hormone replacement treatment. Contrarily, the incidence of breast cancer in women under the age of 45 has remained steady.

For this study secondary data has been collected. From the website of KSE the monthly stock prices for the sample firms are obtained from Jan 2010 to Dec 2014. And from the website of SBP the data for the macroeconomic variables are collected for the period of five years. The time series monthly data is collected on stock prices for sample firmsand relative macroeconomic variables for the period of 5 years. The data collection period is ranging from January 2010 to Dec 2014. Monthly prices of KSE -100 Index is taken from yahoo finance.

Alarming symptoms

Breast cancer is preceded by the expansion of its tissue's hyperplasia (an increase in cell number).It include diffuse alterations in breast tissue and cystic fibrosis, which we have discussed in relation to mastopathy.

Early Signs of Breast Cancer

Every lady should regularly examine herself. It is sufficient to study your chest in the mirror to accomplish this. The following signs and symptoms are typically present:

A. Deformity

B. Inflation

C. A sudden increase or reduction in the size of the breast

D. Nipple or areola zone changes such as erosion, nipple retraction, and discharge that are unrelated to pregnancy and feeding.

E. Skin changes resembling "lemon crust" F. A touchable, painless tight development or thickened area in the mammary gland.
[3]

Breast Cancer Therapy By Class

a) Alkylating agent: cyclophosphamide (nitrogen mustard) .

b) Anti-metabolite: methotrexate (folic acid analogue), 5-fluorouracil &capacitabine (pyramidine analogues).

c) Natural product: vinorelbine (vinca alkaloid), paclitaxel (taxane), doxorubicin (antibiotic)

d) Hormone and antagonist: tamoxifen (anti-estrogen), letrozole & anastrazole (aromatase inhibitors).

e) Miscellaneous: trastuzumab (monoclonal antibody), lapatinib (Protein tyrosine kinase inhibitor). [4]

II. Tamoxifen

When administered in modest dosages, the oral oestrogen antagonist medications tamoxifen is an adjuvant treatment for breast cancer. [5]

The chemical tamoxifen ($C_{26}H_{29}NO$, TAM), which belongs to the class of therapies known as s elective oestrogen receptor modulators (SERMs), has a molecular weight of 371.524 g/mol. [6]
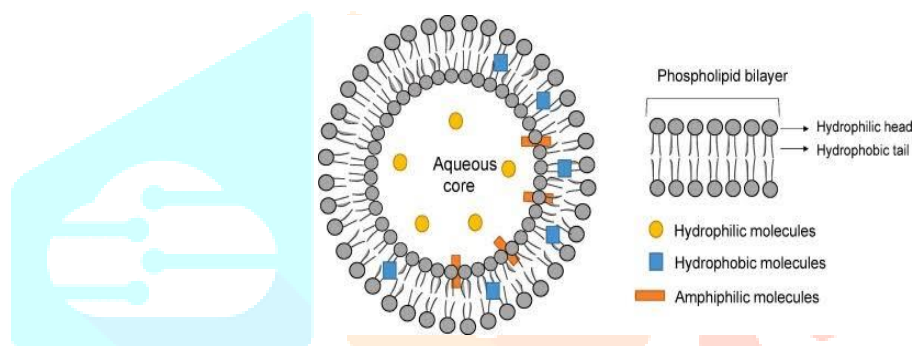

Fig. 1: Chemical Structure Of Tamoxifen

Mechanism Of Action.

Tamoxifen (TAM) is known to have two different mechanisms of action: competing with 17 beta-estradiol (E2) at the receptor site and blocking E2's promotion of breast cancer. Bind DNA during metabolic activation and start the carcinogenesis process. TAM was able to significantly lessen the formation of Ezepoxide when incubated with E2 for epoxidation, as determined by the loss of E's ability to inhibit nuclear RNA synthesis and the decreased binding of [H] labeled Eg .to nuclear DNA. When TAM and estrone (E1) were employed, identical findings were achieved. These findings imply that TAM inhibits breast cancer by competing with E1 and E2 in the epoxidation process. [7]

In many physically regions, tamoxifen has both estrogenic agonist and antagonist actions. It produces both estrogenic and antiestrogenic effects by binding only to oestrogen receptors. As a selective oestrogen receptor modulator (SERM), it is patient specific due to its multiple activities. It opposingly competes with oestrogen for binding sites in the breast tissue, leading to antiestrogenic and anti-cancer actions. Cell cycle decreases through downstream intracellular mechanisms. Consequently, it is categorised as cytostatic. [8]

Dosages of Tamoxifen

- The oral solution (10 mg/5 mL) and tablet (10 mg or 20 mg) forms of tamoxifen are both readily available.
- Although clinical benefit for doses above 20 mg daily has not been proven, it is advised to use 20 to 40 mg daily to treat metastatic breast cancer.
- For high-risk females, breast cancer prophylactic dosage is 20 mg every day for five years.

Contraindication

Patients who have a known allergy to tamoxifen or any other ingredient in its formulation shouldn't take it, and it shouldn't be used with warfarin at the same time. For those using tamoxifen to lower their risk of developing breast cancer who are also at high risk, It should be avoided if the patient has a history of deep vein thrombosis (DVT), pulmonary embolism (PE), or ductal cancer insitu.

Adverse effects

There may be nausea and vomiting. There have been reports of vertigo, tiredness, sadness, irritability, and cerebellar impairment. The smallest total amount of tamoxifen known to have caused retinopathy, a side effect that is known to be dose-dependent. [9]Every year, tamoxifen-using women should get a gynecologic exam. Thrombocytopenia and leucopenia are rare. Different results regarding depression and sexual fluid retention, malfunction, and weight gain.[10]

III. Liposomes

In close colloidal structures called liposomes, aqueous compartments are encapsulated within one or more concentric spheres of lipid bilayers. Because they can transport both hydrophilic molecules (such as carboxyfluorescein and sodium fluorescein) and lipophilic compounds, liposomes have been gaining attention as a drug carrier for drug delivery systems (DDSs) (retinoic acid, and tretinoin). [11]
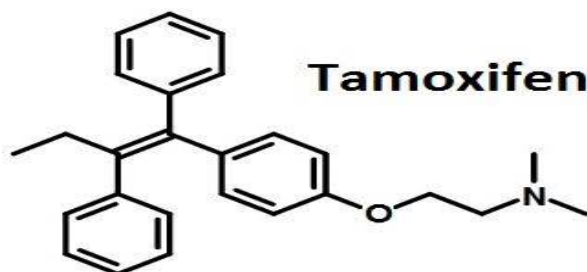

Fig.3: Diagram of Liposome

When soy lecithin, cholesterol, and tocopherol acetate are consolidated, microscopic lamellar structures called liposomes are created. These structures are then hydrated in aqueous media. the liposomes tiny, spherical synthetic vesicles. For the controlled and targeted delivery of anticancer, anti-parasitic, antibacterial, antifungal, antiviral, and ophthalmic treatments, liposomes have received extensive evaluation. Because they may perform as slow releasing vehicles, liposomes are found to be suitable for localising medications used topically at or close to the application site. [12][13]

Liposomes are spherical vesicles used as drug delivery systems. They have a phospholipid bilayer membrane. Liposomes are regarded as the best biological models. membranes, making it possible to use them to convey active substances such as medicines. [14]

METHODS FOR PREPARATION OF LIPOSOMES

To achieve effective drug entrapment, a limited particle size distribution, and longterm stability of liposome products is the major objective of the optimal liposome formulation procedure. The lipid must be hydrated for all liposome production techniques before the particles are size-sorted and the non-encapsulated medication is eliminated. Both passive loading mechanical dispersion methods and active loading methods are employed in the manufacturing of liposomes.

1. **Mechanical dispersion method.**
a. Sonication.
b. French pressure cell: extrusion.
c. Freeze-thawed liposomes.
d. Lipid film hydration by hand shaking, non-hand, shaking or freeze drying.
e. Micro-emulsification.
f. Membrane extrusion. [15]
2. **Solvent dispersion method.**
a. Ether injection (solvent vaporization)
b. Ethanol injection
3. **Detergent removal method (removal of non encapsulated material)** [16][17]

Application of liposome
1) Site-avoidance mechanism
2) Site specific targeting
3) Cancer Chemotherapy and Neoplasia:
4) Arthritis
5) Diabetes
6) Intracellular drug delivery
7) Sustained release drug delivery:
8) Immunological adjuvants in vaccines. [18][19][20]

Advantages of Liposomes

• Liposomes are entirely biodegradable non-toxic, biocompatible, and immunogenic.

• Hydrophobic, amphipathic, and hydrophilic medicines can be administered with this.

• Keep the medicine within its capsule away from the surroundings.

• Improved therapeutic activity of chemotherapeutic drugs can be obtained with decreased toxicity and enhanced stability. via encapsulation in liposomes. This lessens harmful effects that are seen at concentrations comparable to or lower than those essential for the maximal therapeutic efficacy.

• Reduce harmful drug exposure to prone tissues.

Dis-Advantages of Liposomes

• Production cost is high.

• Leakage and fusion of encapsulated drug/molecules.

• Short half-life. [21]

Classification of Liposome [22]

1. Classification of liposome based on size.

Type	Specification	Size
SUVSmall Unilamellar vesicles	Single bilayer	20-100nm
LUV Large Unilamellar vesicles	Single bilayer	100 nm-1 um
IUV Intermediate sized Unilamellar vesicles	Single bilayer	100 nm
GUV Giant Unilamellar vesicles:	Single bilayer	>1μm
MLV Multilamellar large vesicles	Several bilyers	>0.51 m
OLV Oligolamellar vesicles	More than one but not as many as MLV	0.1-11 m

2. Classification of liposome based on method of preparation. [23]

Type	Specification
REV	Single or oligolamellar vesicles made by reverse phase evaporation
MIV-REV	Multilamellar vesicles made by reverse-phase evaporation metho
SPLY	Stable pluri-lamellar vesicle.
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by extrusion technique
DRM	Dehydration-rehydration method

IV. FORMULATION TAMOXIFEN LIPOSOME

1. Preparation of Tamoxifen Loaded Liposomes by Modified Ether Injection Method (MEIM)

In 8 mL of diethyl ether, cholesterol and phospholipids were dissolved, and 2 mL of methanol containing a measured amount of tamoxifen was added. The answer that was reached was 10 mL of phosphate buffer (pH 7.4) hydration solution was injected using a micro syringe at a rate of 1 mL/min while constantly being stirred at 300 RPM on a magnetic stirrer (MS 300 Hot plate Magnetic Stirrer, BANTE instruments, USA). The gradual injection of the lipid solution into the aqueous phase generated rapid ether due to the temperature variations between the two phases, which caused the spontaneous vesicle formation of liposomes. Using a similar process, all of the formulations according to the experimental design were made by adding differed amounts of phospholipids and cholesterol.

2. Preparation of Tamoxifen Loaded Liposomes by Thin Film Hydration Method (TFHM)

Eight millilitres of chloroform were used to dissolve the cholesterol and phospholipid s, and two millilitres of methanol containing a weighted quantity of tamoxifen were added round-bottomed flask. The organic solvents were eliminated using the rotary flash evaporator at 45– 500C and 120 RPM, leaving a thin coating of solid mixture on the flask wall. The dried film is next rehydrated with 20 mL of phosphate buffer solution at pH 7.4 for a predetermined amount of time (about 2- 2.5 hours) while being gently stirred. In order to achieve vesicular suspension, the dispersion was allowed to completely swell the lipid layer by keeping at room temperature unaltered for a period of two to three hours. Finally, by maintaining at 2- 8 0C, the liposome dispersion was maintained. [24][25]

V. EVALUATIONS OF LIPOSOMES

1) **Vesicle shape and lamellarity:**
The form of the vesicles were examined by employing electron microscope.

2) **Particle size and distribution:**
The size was determined via a laser diffraction based analyzer with a minimal power of 5MW31.

3) **Entrapment Efficiency:**
It determines amount and rate of trapping of water soluble substances in aqueous compartment of liposomes.
This can be calculated by a given formula
%Entrapment Efficiency= $\frac{\text{Entrapped Drug}}{\text{Total Drug}} \times 100$

4) **Trapped Volume:**
It is a critical liposomes related metric. It is the volume of aqueous entrap ed lipids per volume of lipids. Between 0.5 and 30 microlitres/micromol are possible ranges.

5) **In vitro drug release:**
The Franz Diffusion cell, which has a 25 mm diameter, can be carried this out. It has a 22.

6) **Percentage yield of liposomes:**
The gathered prepared liposomes were prepared. the amount of medication and other materials used to prepare it was divided by the measured weight. liposomes.

7) **Surface charge :**
The amount of pharmaceuticals contained within the liposomes aids in determining how the drug will behave in a biological system. reservoir compartment that was stocked with buffer with 20% v/v methanol to maintain the sink state. The process of drug encapsulation starts with isolating the drug. the encapsulated drug fraction and the free drug friction. Using the correct detergents, the encapsulated drug fraction is subsequently made to allow the liposomes into the aqueous solution. The methods want to separate the free drug from the sample are:

8) **Percent drug encapsulated:**

The amount of pharmaceuticals contained within the liposomes aids in determining how the drug will behave in a biological system.reservoir compartment that was stocked with buffer with 20% v/v methanol to maintain the sink state. The process of drug encapsulation starts with isolating the drug. the encapsulated drug fraction and the free drug friction. Using the correct detergents, the encapsulated drug fraction is subsequently made to allow the liposomes into the aqueous solution. The methods want to separate the free drug from the sample are:

a) Mini column centrifugation method
b) Protamine aggregates method

9) **Phase behavior:**
Liposomes go through an irreversible phase change at the changeover. The TC is a marker of stable permeability that also identifies an area of drug entrapment. DSC conducts the task.

10) **Drug release rate:**
In vivo assays can be used to calculate the liposomes' rate of drug release, which aids for pre dicting the medication's pharmacokinetics and bio-availability. However, it is discovered that in vivo research are more thorough. The study uses liposomes that contain the tracer insulin. Because it is only released within the ECF as well as experiences quick renal excretion of the face tracer combined to the degradation rate constant of the released from the liposomes tracer, this insulin is suggested.

11) **Particle Size**
These can be determined by the following method
a) Laser light scattering
b) Transmission electron microscopy [26]

12) **Surface Charge**
The head group's composition is what gives the liposomes' surface a passive, negative, or natural charge. The velocity and extent of liposomes can be controlled by their surface charge. distribution in living organisms, as well as contact with the target cells. The method used to assess surface charge is anticipated on MLV free flow electrophoresis. It makes use of a cellulose ester plate immersed in a buffer of pH 8.8 sodium borate. The plate receives around moles of lipid samples and is then exposed to electrophoresis at 4°C for 30 min. The surface charge of the liposomes determines how they divide into two. [27]

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