ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Review Article*
www.ijptonline.com

STEALTH LIPOSOMES AS NOVEL DRUG DELIVERY SYSTEM FOR ARTHRITIS

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Received on 18-01-2017 *Accepted on: 12-03-2017*

Abstract

The aim of this review work is to investigate the efficacy of stealth liposomes as novel drug delivery system in arthritis. Novel drug delivery system has a major advance to solve the problems related to the drug release at specific site. There are various approaches for delivering the drug to the site. One such approach of novel drug delivery system is stealth liposome. Arthritis is the most common form of disability affecting joints. The slow efficacy in the treatment of rheumatoid arthritis and osteoarthritis has suggested the growing need of stealth liposomes as novel drug delivery system in arthritis. Stealth liposomes are polymer coated liposome to camouflage drug surfaces. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment, controlled release and increased solubility. This review work gives brief information about rheumatoid arthritis, osteoarthritis, liposomes, stealth liposomes, method of preparations and application of stealth liposomes in drug delivery.

Key words: Liposomes, Novel drug delivery system, Osteorthritis, Rheumatoid arthritis, Stealth liposomes.

Introduction

Over the last decades, important progress in pharmaceutical field led to the discovery and design of modern pharmaceuticals and to the development of successful strategies to improve the clinical activity of new and old drugs. In the progress of the medical sciences, drugs become more specific and the doses to be used become smaller [1]. The recent advances in novel drug delivery system are vital research areas in nanotechnology which aim to enhance the safety and efficacy of the drug molecule. The novel drug delivery system aims in developing new ideas such as control of pharmacokinetics, pharmacodynamics, non- specific toxicity, immunogenicity, biorecognition and efficacy of drugs.

Various drug delivery system and drug targeting systems are currently under development with a goal on promoting the therapeutic effect of a drug and minimizing toxic effect. The very slow progress in the efficacy of the treatment of severe diseases such as arthritis has suggested a growing need of novel drug delivery system [2].

In NDDS, nanotechnology is theoretically able to achieve various objectives, such as optimizing drug delivery into a specific area of the body and protecting active compounds until they reach their pharmacological target. Most nanosystems that encapsulate drugs are solid colloid particles expected to penetrate through smaller capillaries before being subsequently up taken by cells, thus theoretically allowing drug accumulation into target tissues. Relatively lesser investment of time and money in novel drug delivery system could lead to higher margins of profit [3]. The most common inflammatory diseases such as rheumatoid arthritis (RA) and osteoarthritis experience limited treatment success with conventional therapy. Arthritis may result in a shortened life span [4].

Arthritis

Arthritis is a term used to describe any disorder that affects joints. Arthritis is the most common form of disability. There are over 100 types of arthritis. The most common forms are osteoarthritis and arthritis. Other types of rheumatic diseases include gout, lupus, fibromyalgia and septic arthritis. When arthritis is left uncontrolled, the patient may experience joint deterioration, severe disability, decreased quality of life, the onset of comorbidities and premature mortality [5].

Most Common Types of Arthritis:

Adult Rhumatoid Arthritis

Rheumatoid arthritis is a chronic autoimmune disease characterized by inflammation of the lining or synovium [fig.1]. Autoimmune diseases are illnesses that occur when the body's tissues are mistakenly attacked by their own immune system. Rheumatoid arthritis occurs mostly in people aged 20 and above. RA is a disease with worldwide prevalence of approximately 0.5% to 1% among adults.

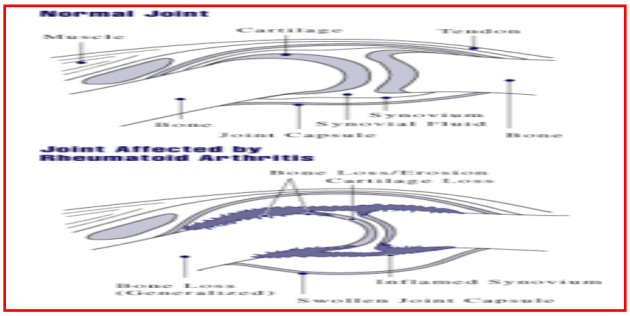

Fig.1 Rheumatoid arthritis affecting joints.

Juvenile Rheumatoid Arthritis (STILL'S DISEASE):

Considered a major crippler of young children. Usual onset is before 7 years of age.

Signs and symptoms of rheumatoid arthritis

The signs and symptoms of rheumatoid arthritis are the following

- Fatigue
- Joint pain
- Joint swelling
- Joint redness
- Joint warmth
- Joint stiffness
- Joint tenderness
- Loss of joint range of motion
- Many joints affected (polyarthritis)
- Limping
- Joint deformity
- Both sides of the body affected (symmetric)[*www.medicinenet.com/rheumatoid arthritis early symptoms /article. htm (20dec 2016)]*.

Osteoarthritis

Osteoarthritis is a common degenerative disorder of the articular cartilage affecting about 3.8% of people along with hypertrophic bone changes. Damage from mechanical stress with insufficient self repair by joints is believed to be the primary cause of osteoarthritis. Other risk factors of osteoarthritis are past trauma, genetics, obesity, and advancing age *[https://en.wikipedia.org/wiki/osteoarthritis[dec20 2016]*

Signs and symptoms of osteoarthritis [6]

- Pain
- Joint effusion
- Bone spurs
- Tenderness
- Stiffness
- Inelasticity
- Bone crunching
- Muscle atrophy

Ankylosing Spondylitis:

Affects the axial skeleton and large peripheral joints of the body. Most prevalent in males with the age of onset ranging from 20 to 40 years of age. Common symptoms include recurrent back pain and early morning stiffness.

Role of Nsaids in Arthritic Pain Management

NSAIDs are used as effective therapy for pain management to relieve pain, swelling (inflammation), and joint stiffness caused by arthritis. NSAIDs mainly work by blocking the effect of chemicals called cyclo-oxygenase (COX) enzymes thereby decreasing prostaglandins synthesis. Prostaglandins play a role in pain and inflammation which is a group of naturally occurring fatty acids. By blocking the effect of COX enzymes, fewer prostaglandins are produced, leading to pain and inflammation cessation6s. The safety of NSAIDs has been closely monitored by regulatory authorities across the globe [7].

Effective drug delivery of NSAIDS can be done by novel drug delivery system such as Liposomes.

Liposome: The Magic Bullet

Liposomes have been widely used as drug carriers. Liposomes are artificially prepared self-assembled vesicles composed of a lipid bilayer which forms a closed shell surrounding an internal aqueous phase. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body [8]. Liposomes are a beau ideal in smart delivery of drugs. Liposomes can vary in size, ranging from the smallest vesicle (20 nm) to liposomes that are visible under the light microscope with a diameter of 1µm or greater, equal to the dimensions of living cells. Liposome is also flexible in their size and enables water soluble and water insoluble materials to be used together in a formulation without the use of surfactants and emulsifier [9]. .When phospholipids are placed in water and sufficient energy is provided from sonication, heating and homogenization result in the arrangement of lipids and formation of bilayered vesicles. It is due to the CMC of phospholipids in water, means the concentration of lipids in water above which the lipids form micelles or bilayer structures rather than remaining as monomers in solution. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of lipid crystalline bilayers become fluid and swell [Fig.2]. The hydrated lipid sheets detach during agitation and self close to form large liposomes to prevent interaction of water with the hydrocarbon core of the bilayer at the edges [10, 11]. Liposomes can be classified according to their lamellarity (uni-, oligo-, and multi-lamellar vesicles), size (small, intermediate, or large) and preparation method (reverse phase evaporation vesicles, VETs).

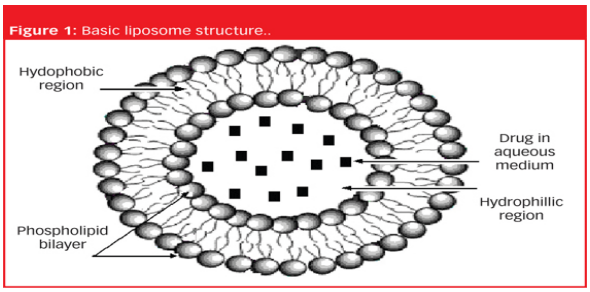

Fig.2. Liposomes formed by phospholipids in water.

The choice of liposome preparation method [Fig.3] depends, on the following parameters [12].

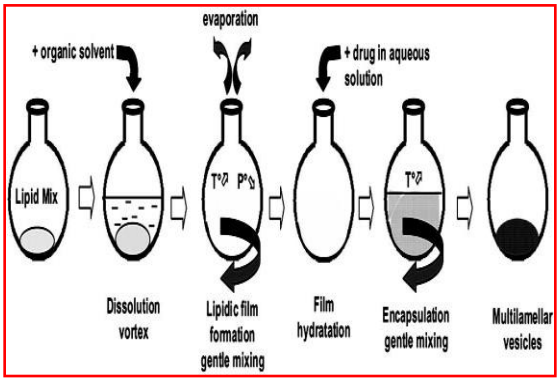

Fig. 3.Preparation of Multilamellar vesicles by standard thin film method.

1. The physicochemical characteristics of both the material entrapped and liposomal ingredients.
2. The nature of the medium in which the lipid vesicles are dispersed.
3. The effective concentration of the entrapped substance and its potential toxicity.
4. Any additional processes involved during application/delivery of the vesicles.
5. Optimum size, polydispersity and shelf-life of the vesicles for the proper application.
6. Batch-to-batch reproducibility and large-scale production of safe and efficient liposomal products.

Advantages of Liposomes [13, 14]

- It should be biodegradable, biocompatible, and flexible
- Decreased toxicity
- Enhanced activity of drugs against intracellular pathogens.
- Liposomes can be target selective
- Improvement and control over pharmacokinetics and pharmacodynamics.
- It can carry both water and lipid soluble drugs.

- The drugs can be stabilized from oxidation.
- It can improve the protein stabilization.
- It provides controlled hydration.
- Stabilization of entrapped drug from hostile environment.
- The therapeutic index of drugs is increased.
- Site avoidance therapy.

Disadvantages of Liposome [15, 16]

Some of the problems limiting the manufacture and development of liposome are the stability issues, batch to batch reproducibility, sterilization method, low drug entrapment, particle size control, oxidation of phospholipids, production of large batch sizes and short circulation half-life of vesicles.

- **Short shelf life**

Liposomes are thermodynamically unstable system which easily aggregate and tend to fusion. In order to improve the stability of liposome, several parameters should be strictly controlled such as choice of buffer, addition of cholesterol/sphingomyelin in liposome film, and control of particle size and distribution. Two factors play a major role in the stability of liposomes namely, chemical and physical degradation. The chemical degradation of liposomes is attributed to oxidation and hydrolysis. Physical processes such as aggregation/flocculation and fusion/coalescence that affect the shelf life of liposomes can result in loss of liposome associated drug and changes in size. As coalescence is an irreversible process the original liposomes cannot be retrieved.

- **Organic Solvents Residue**

It is unavoidable to use organic solvents in liposome preparation which may affect the stability of liposomes and also harmful to human body. Therefore, the residue of organic solvents should be strictly controlled by proper preparation methods.

- ***In vivo* instability of liposome**

Liposomes can be administrated via various routes, including parenteral and non-parenteral. Intravenous administration was the most common route, since it can demonstrate the targeting properties of liposomes. Upon intravenous

administration, liposomes are diluted by large amount of blood, which would affect the *in vivo* stability and the fate of liposomes.

Liposomes are recognized by the mononuclear phagocytic system (MPS), after interaction with plasma proteins they are cleared from the bloodstream by MPS and trapped by the reticuloendothelial system (RES) in which macrophage-like cells reside [Fig4]. RES is composed of liver, spleen, and bone marrow.The binding of plasma proteins, such as complements, immunoglobulins, and fibronectin enhances the trapping of liposomes by macrophages. Since drug distribution implies a very rapid clearance, high drug dosages had been used generating undesirable side effects. Also short circulation half-lives have appeared insufficient to reach many tissues. Liposomes can be destabilized by certain cell membrane components leading to the release of encapsulated drugs extracellularly causes short circulation profile.

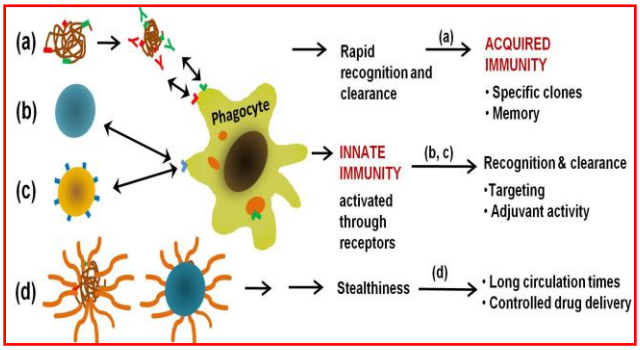

Fig.4 Interactions of therapeutic formulations with the immune system after intravenous administration.

a) Drugs may be released in a free form, b) inside nanoparticles, c) in nanoparticles engineered for targeting immune cell, d) in a camouflaged polymer-grafted form

Stealth Liposomes: To Live Happy, Live Hidden

Stealth liposomes utilize the same concept of stealth bombers that escape detection by radars in World War II. We need such a newly designed delivery system which delivers the killer drugs deep inside tumor cells and destroy them, just like the stealth bombers did. The liposomes are camouflaged to fool phagocytosis by ignoring them. Stealth liposomes were introduced which can largely avoid detection by immune system and were shown to have blood circulation time for several days [17].

Basically two approaches have been considered for avoiding or reducing RES trapping of liposomes. The first strategy was to mimic the erythrocyte membrane using some glycolipids such as ganglioside GM1 and phosphatidylinositol, which has been proved to be capable to decrease the clearance rate of liposomes, however, none of them reached clinical

stage. The second strategy is to modify the liposome surface with hydrophilic polymers, such as polyethylene glycols (PEGs) to achieve long circulation. Such hydrophilic polymers possessed flexible chains and provided an additional surface hydration layer on the conventional liposome and inhibited the binding of plasma opsonins to liposome surface, which resulted in escaping uptake by RES and prolonged circulation period[18]. To phagocytes, this molecular "cloak" of water of hydration makes the PEGylated liposomes look like little watery blobs rather than something edible, so they tend to leave them alone [Fig.5].

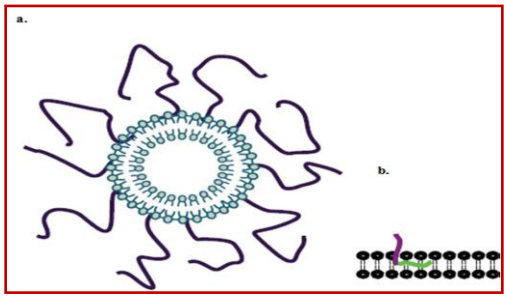

Fig.5 Stealth Liposomes.

a) Liposomes with stealth properties due to the existence of PEGylate lipids, b)The structure of the block copolymer grafted liposomal membrane.

Formulation of Liposomes and Stealth Coating

1. Membrane Forming Components

Phospholipids: As main components of cellular membrane, phospholipids have excellent biocompatibility [19]. Phospholipids are known for their amphiphilic structures. The amphiphilicity offers phospholipids with self-assembly, emulsifying and wetting characteristics. Phospholipids have a tendancy to form liposomes, which can be employed as the drug carriers. Phospholipids are lipids containing phosphorus, a polar portion and non-polar portion in their structures.

Five groups of phospholipids are used for liposomal preparation:

- Phospholipids from natural soures
- Modified natural phospholipids
- Semisyntheyic phospholipids
- Fully synthetic phospholipids
- Phospholipids with nonnatural head groups

Examples of phospholipids are:

o Phosphatidyl choline (Lecithin) – PC

o Phosphatidyl ethanolamine (cephalic) – PE

Sphingolipids: Sphingolipids are also an important component of animal cell membranes. Sphingomyelins (SM) are capable of forming intermolecular and intramolecular hydrogen bonds. The range of phase transition temperature (*Tc*) of all naturally occurring SMs is 30–45 °C[20]. Numerous observations have shown that SM and cholesterol have a very strong interaction. When compared with the non-saturated PC/cholesterol bilayer, the SM/cholesterol bilayer has higher compressibility and lower permeability to water. This is due to the higher saturation of the acyl chain of SM which leads to stronger interaction with steroid nucleus.[18] Natural gangliosides(GM1)class of sphingolipids are included in liposome formulations to provide a layer of surface charged groups and to prolong the lifetime of liposomes in the blood thus prevent their uptake by the reticuloendothelial system.

2. Membrane Additives *(sterols)* [21,22]

Cholesterol, another important component of cell membranes, does not form bilayers by itself, but will dissolve readily in the phospholipid bilayer.Cholesterol is called mortar of bilayers. n. It is able to control the membrane permeability by inducing conformational ordering of the lipid chains. They are included in liposomes for

- Reducing the fluidity and microviscosity of the bilayers.
- Decreasing the permeability of the membrane to the water molecules.
- To stabilize the membrane in the presence of the biological fluids such as plasma.

Eg: 6-carboxyfluorescin entrapped in cholesterol rich [egg PC-CH 7:7 molar ratio] liposomes exhibited a slow linear clearance with about 70% of the dose being present in the blood at 60 minutes.

3. **Charge Inducers** [23, 24]

These are added to reduce the aggregation of the liposomes after preparation. Since it is a well-known fact that negatively charged and positively charged liposomes are more rapidly uptaken by the reticulo-endothelial system as compared to neutral liposomes, charge inducers are used to overcome this problem.Stearylamine and Dicetylphosphate are the positive and negative charge inducers, which creates an electrostatic repulsion between the adjacent vesicles thus prevents from the formation of micelles and forms a highly stable suspension.

4. Stealth Polymers [25, 26]

Large numbers of polymers are used to offer surface hydrophilicity and flexibility to make stealth coating to impede phagocytosis.

- **Polyethylene Glycol (PEG)** is mostly used in the production of Stealth liposome. PEG is suitable for biological applications because it is soluble in water and has low intrinsic toxicity. It directly adheres to or covalently bonds to the outer surfaces of the liposomes forming long circulating liposomes. PEG possesses the following properties as an ideal polymer:

- Biocompatible and biodegradable.
- Degradation product is non-toxic
- Does not produce inflammatory response
- Degradation time is within a reasonable period of time
- Completely or partially invisible to mononuclear phagocytic system (MPS)
- Permeable to lipid bilayer and blood-brain-barrier.

- **Polyacrylamide (PAA)** is a synthetic polymer derived from acrylamide monomer. It is highly hydrophilic and used as an alternative to polyethylene glycol. Polyacrylamide is stable over wide pH intervals (pH 3–11), simple and economical. It is also used as a carrier for other bioactive macromolecules and cells to produce the desired effects.
- **Poly vinylpyrrolidone** (PVP) or **povidone**, is a water-soluble polymer. The soluble PVP grades are also useful for preparing solid solutions and dispersions because of their good hydrophilization properties, universal solubility and ability to form water soluble complexes. PLA-PVP micelles and microspheres as well as PVP-gelatin hydrogels and PVP have been studied in respect of their roles in aiding good formulations. PVP hydrophilizes the individual solid particles and sterically separate them.
- **Poly (2-methyl-2-oxozoline) and poly (2-oxazoline) form** an important class of polymers used for extended or long circulation time and decreased uptake by mononuclear phagocyte system (MPS).

Hyaluronic acid (HA), **Dextran, Chitosan**,**Polysorbates** are other important polymers used in the production of stealth liposomes.

Stealth Liposomes Manufacturing Techniques [27]

There are three ways to modify a liposome surface with lipopolymers:

- ❖ incorporating an amphiphilic conjugate of the polymer during liposome formation (pre-insertion)
- ❖ inserting the polymer conjugate onto the surface of pre-formed liposomes (post-insertion)
- ❖ post-modification by chemically reacting a polymer to the exposed functionalities on the liposome surface.

Mechanism of Stealth Liposomes [28]

Since, liposomes cause aggregation in the blood by their mutual reaction (Vander Waals interaction or hydrophobic interaction) with various blood plasma proteins and are captured by the reticuloendothelial system (RES), selected delivery of the liposomes to target tissues or cells become very difficult. For example, kupfer cells in the liver or fixed macrophages in the spleen take up the liposomes before they can reach their intended target. Also the liposomes are subjected to electrostatic, hydrophobic and Vander Waals interactions with plasma proteins These interactions result in destabilization of the liposomes leading to rapid clearance of the vesicles from circulation, often before reaching their target. The main feature of long circulating liposomes is ability to extravasate at body sites where the permeability of the vascular wall is increased. The PEG-coated liposomes (stealth or sterically stabilized liposomes) have escaping capability and steric stabilization mechanism held responsible for the induction of long circulation times. Magic gun approach-particulate drug carriers PEGylated liposomes avoid detection and shattering by phagocytes by virtue of their cloaks of hydrated PEG (polyethylene glycol) molecules. They increases the bioavailability of drugs or supplements by passing the digestive tract and then to minimize any potential toxicity or side effects of these molecules by remaining in the circulation for a prolong time and releasing their content slowly [Fig.6].

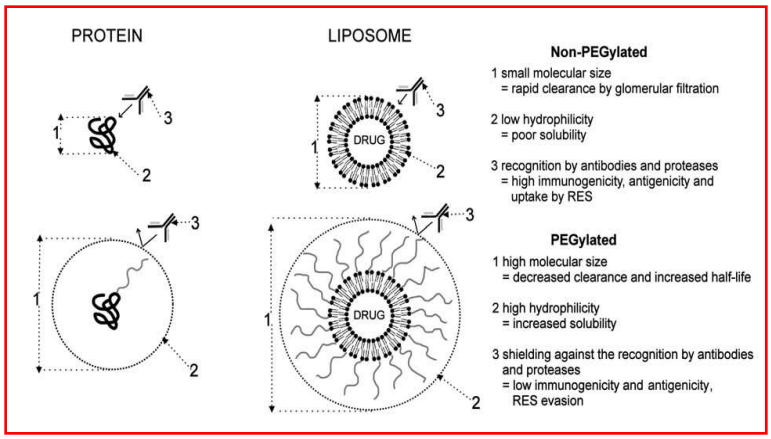

Fig.6 Main advantages for PEGylation of proteins and liposomes.

Evaluation of Liposomes [29]

The liposomal formulation and processing for specified purpose are characterized to ensure their predictable in vitro and in vivo performance [Table.1]. The characterization parameters for the purpose of evaluation could be classified into three categories i.e. physical, chemical and biological parameters. Physical characterization evaluate parameter includes size, shape, surface, and drug release profile. Chemical characterization includes studies in establish the purity and potency of various lipophillic constituents. Biological characterizations parameters are helpful in establish the safety and suitability of formulation for therapeutic application.

Table.1 Different methods of characterization of liposomes.

Characterisation parameter	**Instrumental analysis**
Phospholipid hydrolysis	HPLC/TLC
Drug release	Diffusion cell/Dialysis
Vesicleshape/surface morphology	Tem/SEM
Phase behaviour	DSC/ Freeze fracture electron microscopy
Lamellarity	P^{31}NMR
Electrical surface potential	Zeta potential measurement
Animal toxicity	Monitoring survival rats

Conclusion [30]

Stealth liposome can play a vital role in drug delivery, more efficiently and on a target based approach. As the stealth liposome provides definite advantages, it is a better choice for drug delivery in arthritis. The current advancements in novel drug delivery systems are helpful in overcoming challenges offered by the traditional systems. It also provides solutions to enormous questions that were remained unanswered.

Acknowledgement: We express our utmost gratitude and thankfulness to staff and management of Nazareth college of Pharmacy, Othera, Thiruvalla [India] for providing support and providing other necessary facility such as internet surfing, library and other support for completing the article.

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