The Potential of Liposomal Drug Delivery for the Treatment of Inflammatory Arthritis

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Objective: To review the use of liposomes as a delivery agent in inflammatory arthritis.

Methods: The literature on liposomes and liposomal drug delivery for the treatment of inflammatory arthritis was reviewed. A PubMed search of articles in the English-language journals from 1965 to 2007 was performed. The index words used were as follows: "rheumatoid arthritis," "liposomes," and "targeted delivery." Papers identified were reviewed, abstracted, and summarized.

Results: Liposomes have the capacity to be used as delivery and targeting agents for the administration of antirheumatic drugs at lower doses with reduced toxicity. In other areas of medicine, the pace of progress has been rapid. In the case of infectious diseases and cancer, liposomal drug delivery has progressed and developed into commercially viable therapeutic options for the treatment of fungal infections (amphotericin B), or metastatic breast cancer and Kaposi sarcoma (doxorubicin, daunorubicin), respectively. In arthritis, the efficacy of prednisolone-loaded long-circulating liposomes is currently being evaluated in a phase II clinical trial. Liposome's application to arthritis is still in its infancy but appears promising as new patents are filed. With improvements in liposomal formulation and targeted synovial delivery, liposomes offer increased therapeutic activity and improvement in the risk–benefit ratio.

Conclusion: Recent research into synovial targets and improved liposomal formulations continues to improve our capacity to use liposomes for targeted delivery. With time, this approach has the potential to improve drug delivery and reduce systemic complications.

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Keywords: *liposomes, rheumatoid arthritis, drug delivery, targeted delivery*

With advances in biotechnology, new drugs and novel delivery systems are constantly in pursuit. Recent interest in liposomes has focused on devising ways to improve delivery methods, thereby reducing toxicity and consequently improving the therapeutic index.

The field of liposomes is complex and involves many areas of science including chemistry, biology, biophysics, and physics. Since the discovery of classic liposomes over 40 years ago by Alec Bangham, liposomes have been extensively investigated as potential carriers of drugs and biologically active molecules (1). The development of second-generation sterically stabilized liposomes in which the liposome surface is coated with polyethylene glycol (PEG) allows the liposomes to evade phagocytosis by the reticuloendothelial system (RES) and thereby extend the liposomes' circulation time. In addition, selective delivery of encapsulated drugs to specific target sites (eg, synovium, tumors) has become possible by covalent attachment of antibodies/antibody fragments or ligands to the liposomal surface.

These advances have led to a resurgence of liposome research, resulting in their renewed application to clinical medicine and a re-evaluation in the area of drug delivery. The application of liposomes has been previously reviewed (2-6) and much of the literature pertains to experimental animal models with little written about liposomal

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Abbreviations	
AIA	Antigen-induced arthritis
B	Boron
CIA	Collagen-induced arthritis
COMP	Cartilage oligomeric protein
CD	Cluster of differentiation
DMARD	Disease-modifying antirheumatic drug
GC	Glucocorticoids
GPI	Glucose-6-phosphate isomerase
HIV	Human immunodeficiency virus
HVJ-liposomes	Hemaglutinating virus of Japan-liposomes
IL	Interleukin
K	Lysine
Lf	Lactoferrin
LUV	Large unilamellar vesicles
MLV	Multilamellar vesicles
MPS	Mononuclear phagocyte system
MTX	Methotrexate
NSAID	Nonsteroidal anti-inflammatory drug
ODN	Oligodeoxynucleotide
PC	Phosphatidylcholine
PEG	Polyethylene glycol
pHLIPs	pH (low) insertion peptide
PTD	Protein transduction domains
R	Arginine
RA	Rheumatoid arthritis
SLX	Sialyl Lewis X
SOD	Superoxide dismutase
SUV	Small unilamellar vesicles
TNF	Tumor necrosis factor
TRX-20	3,5-dipenta-decycloxy-benzamidine hydrochloride
VEC	Vascular endothelial cells

application in the clinical setting. The aim of this review was to provide the reader with a wider view of the concepts and applications of liposomes, and how they might apply to the treatment and management of rheumatoid arthritis (RA) and/or other inflammatory arthritis through targeted drug delivery systems.

Many of the current drugs used to treat rheumatic disorders are effective (eg, nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids). Recent studies highlight glucocorticoids' (GC) effectiveness in reducing inflammation and slowing joint damage, especially when patients are treated at an early stage of disease propagation (7). However, the toxicity associated with these drugs is also well documented particularly with long-term use and administration (8). A possible solution to reducing toxicity is to achieve high systemic concentrations of the drug at the active site while minimizing exposure of the drug systemically and to unaffected tissues. Intra-articular administration of the drug has been one option and adequately complements other forms of treatment. However, a large number of limitations exist and some of these include the need for repeated joint needling, the need to inject large number of joints, or the size and accessibility of involved joints (9). Targeted delivery is therefore an attractive option.

Currently, a clinical trial concerning the therapeutic effect of prednisolone disodium phosphate loaded long circulating liposomes compared with a single intramuscular administration of free methylprednisolone is in the final stages of a phase II study for the treatment of RA (Barrera, P. Radboud University, Clinical trials identifier: NCT00241982). Funk and coworkers filed a patent (WO/2007/134819) reporting cationic liposomal preparations for the treatment of RA, based on the capability of cationic liposomes in targeting the vascular endothelium of mice joints and consequently alleviating arthritis (10). Despite the fact that at present there is a paucity of information relating to liposomes in clinical trials for the treatment of RA, this should not negate their potential future use. This may merely reflect the present lack of focus and investment by pharmaceutical companies in this field. Liposomes for the treatment of various other diseases are listed in Table 1.

METHODS

This article outlines the basic features of liposomes and the current management of inflammatory arthritis with an emphasis on the mode of delivery through the use of liposomes. Literature on delivery strategies dating from 1956 to 2007 were searched using PubMed from the National Library of Medicine and reviewed. The index words used were "rheumatoid arthritis," "liposomes," and "targeted delivery."

RESULTS

General Overview of Liposomes

Liposome Structure and Characteristics

Liposomes are spherical vesicles or cavities made up of phospholipids that usually, but not by definition, contain a core of aqueous solution (11). They are well known for their ability to protect encapsulated therapeutic agents, extend their duration of action, enabling effective intracellular delivery (12,13). Liposomes are derived from naturally occurring, biodegradable, and nontoxic lipids that form a closed bilayer sphere when the hydrophobic phospholipid molecules come into contact with the aqueous environment (5). This allows the closed sphere to encapsulate water or soluble drugs within the central compartment, while water-insoluble drugs can be incorporated in the hydrophobic region of the membrane (Fig. 1).

Liposomes vary in size ranging from 30 nm to several micrometers, phospholipid composition, and surface characteristics. These features can be modified to suit specific applications. Single bilayer lipid structures are commonly referred to as small unilamellar vesicles (SUV: 20-100 nm) or large unilamellar vesicles (LUVs: >100 nm) (6). Large multi-lamellar vesicles (MLV) or multi-vesicu-

lar vesicles have more than one bilayer and range in size from a few hundred nanometers to several microns (14-16) (Fig. 2). Different types of lipids have varying functions, and thus, the choice of lipid [eg, phosphatidylcholine (PC), phosphatidylethanolamine, or 1,2-dioleoyl-3-trimethylammonium-propane] can determine the type of liposome produced. In addition to the liposome surface being changed by the selection of lipids, the amalgamation and linkage of glycoproteins and synthetic polymers to the bilayer can also alter the biophysical characteristics of liposomes (17-19).

Preparation Methods

Depending on the preparation process, various liposomes (SUV, LUV, MLV) can be produced by different methods, suggesting several mechanisms play a role in their formation. The fundamental component in forming liposomes is that lipid molecules must be introduced into an aqueous environment above the transition temperature of the lipids. It is widely accepted that dry lipid films spontaneously form MLVs on introduction of an aqueous substance (Fig. 3). However, Lasch and coworkers state that their formation only takes place on mechanical agitation (shaking, swirling, pipetting, or vortexing). This causes the thin lipid tubules to break and reseal the exposed hydrophobic edges, resulting in the formation of the liposome. The most popular and simplest method of MLV preparation is the thin-film hydration procedure (20).

Table 1 Liposomal Drugs and Their Applications

Drug	Application
Amikacin	Bacterial infections
Adriamycin (Doxorubicin)	Stomach cancer/other cancers
Ampicillin	Listeria monocytogenes
Annamycin	Kaposi's sarcoma, breast cancer, leukemia
Amphotericin B (Ambisome)	Systemic fungal infection
All-trans-retinoic acid	Acute promyelocytic leukemia, lymphoma, prostate cancer
Muramyl dipeptide	Immunostimulator
Clodronate	Macrophage suppression
Cyclosporin	Immunosuppressor
Ciprofloxacine	*Pseudomonas aeruginosa*
Cis-diaminodichloro-platinum(II)	Cancers
Chloroquine	Malaria
Cu/Zn superoxide dimutase	Ant-inflammatory
Daunorubicin	Cancers
Ganciclovir	Cytomegalovirus retinitis
Interleukin 2	Immunostimulant
Mitoxantron	Colon cancer
Methotrexate	Cancers
Nystatin	Systemic fungal infections
Pentostam	Leishmoniasis
Cisplatin (PLATAR)	Mesothelioma
Lurtotecan (NX 211)	Cancers
1-β-D-Arabino-furanozidecytozine	Leukemia
Ciprofloxacine	*Pseudomonas aeruginosa*
Lipid A	Immunoadjuvant
$Na_3(B_{20}H_{17}NH_3)$	Cancers
Prostaglandin E1	Anti-inflammatory
Ribavirin	Herpes simplex
Streptozotocin	Lymphocte activator
Suramin	Trypanosomes

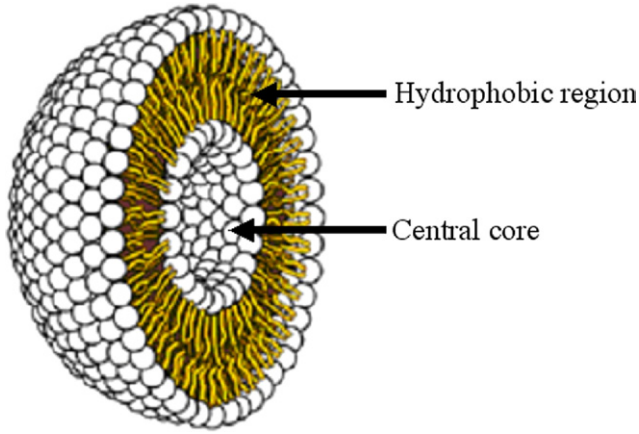

Figure 1 Schematic view of liposome. Cross-sectional view of a liposomal structure that can be formed by phospholipids in an aqueous solution. (Color version of figure is available online.)

Types of Liposomes

Liposomes presently classified as conventional are negatively charged or neutral. Cationic liposomes impose a

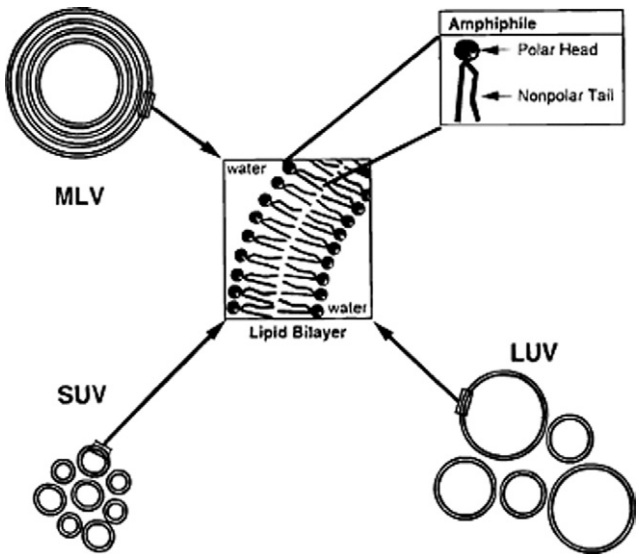

Figure 2 The schematic illustration of liposomes based on different size and number of lamellae. SUV: small unilamellar vesicles; MLV: multilamellar vesicles; LUV: large unilamellar vesicles. Figure reproduced with permission from Avanti Polar Lipids, Inc.

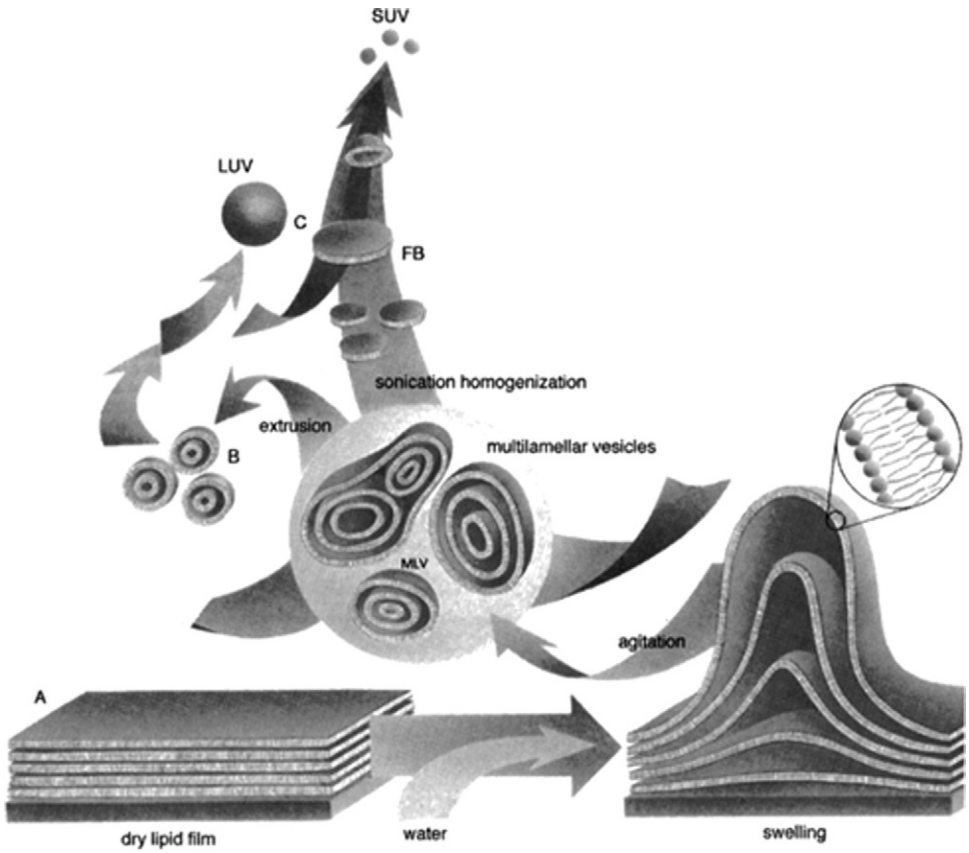

Figure 3 Preparation of liposomes. (A) Lipids are combined and dried on a rotary evaporator and freeze dried. On hydration unilamellar or multilamellar liposomes are formed. (B and C) The size of the liposome can be altered depending on the technique used. Figure reproduced with permission from Avanti Polar Lipids, Inc.

positive surface charge; sterically stabilized long circulating (stealth) liposomes increase circulation time (Fig. 4). The targeting agents can be antibodies (immunoliposomes) or other specific ligands (eg, peptides) that are attached to the liposome surface either with or without a linker (6).

Conventional Liposomes

The first type of liposome, composed of egg PC and cholesterol, is commonly referred to as a conventional liposome. When given intravenously, conventional liposomes are quickly coated with plasma proteins, enhancing their phagocytosis by RES cells. This results in rapid removal from systemic circulation. Although this has been advantageously exploited in the treatment of parasites that reside in the liver and spleen (6,21), their very short circulating half-life has deterred the initial interest as a delivery vehicle.

Modification of liposomal surfaces with proteins, peptides, antibodies, carbohydrates (eg, sialoglycoprotein fetuin), and polymers has led to prolonged circulation time (22,23). Although other ligands, such as derivatives of dicarboxylic acids and dextrans, improve circulation time, arguably one of the most important breakthroughs in liposomal delivery came with the understanding of the potential uses of the linear synthetic polymer, PEG (21,24).

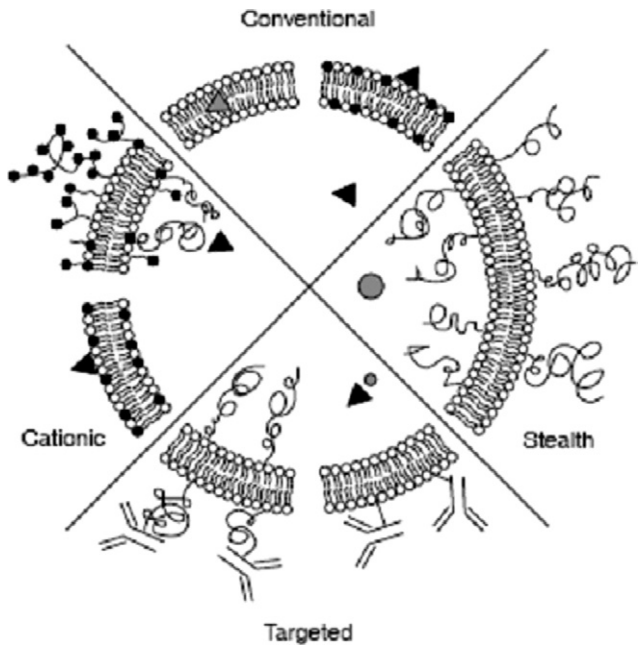

Figure 4 Four major types of liposomes. Conventional, stealth, immunoliposomes, and cationic liposomes. Figure reproduced with permission from Lasic DD. Sci Med 1996; 3:34-43.

Long Circulating "Stealth" Liposomes

PEG increases the hydrophilicity of the liposome, resulting in reduced interactions with plasma proteins and lipoproteins (25-27). Steric stabilization results from an accumulation of highly hydrated surface PEG groups that prevent interactions with molecular and cellular biological components (28). Liposomal pegylation serves two important functions. First, to increase the bioavailability of drugs, and second, it enables slow release of their load so that side effects and toxicity are kept minimal (5,29,30).

Other polymers besides PEGs include polyacrylamide, polyvinyl alcohol, and polyvinylpyrrolidone. These are often referred to as "steric protectors" for their ability to protect the liposome from elimination by the RES (31-34).

One of the most important features of stealth liposomes is their ability to extravasate at sites where there is high permeability at the vascular walls. As sites of infection and inflammation generally have increased capillary permeability, the above-mentioned features of the PEG groups are favorable (6).

Cationic Liposomes

These liposomes are useful as a delivery system for genetic material (35-38). The cationic lipid components neutralize negatively charged DNA, forming a more compact structure. The resultant DNA–lipid complex provides protection and promotes cellular internalization and expression of the plasmid (6).

Immunoliposomes

Immunoliposomes are able to actively target and recognize specific cells and organs of the body due to the nature of their structural design. Specific cell recognition is achieved by the presence of antibodies or antibody fragments on the surface of liposomes (13). The selection of the target antigen, function of the antibody, and type of linker used (eg, PEG) are factors that require examination (39). An example of this is targeting the activated aggressive cluster of differentiation 4 (CD4) T cells that initiate the onset of adjuvant induced arthritis (AIA), by drug-containing anti-CD134 targeted liposomes. The end result is an alteration and improvement in the course of AIA (40).

In summary, the bio-distribution and circulation times of liposomes can be influenced by measures such as particle size, lipid composition, surface charge, hydration, sensitivity to pH changes, bilayer rigidity/fluidity, and the binding kinetics of liposomes to cell surface receptors (16,18,41). To improve stability and targeting potential, surface-modified liposomes have been used. These range from simple molecules (monosaccharides, folic acid, and transferrin) to more complex structures such as antibodies and other chemical entities. Cationic liposomes can undergo lipid mixing with cellular membranes to deliver loads such as DNA.

Liposomes in Infections

Traditionally, liposomes have been used in the realm of the textile and cosmetic industry. However, their application in medicine is now more recognized and they are used as vaccines and diagnostic and therapeutic agents. A plethora of research has been performed with liposomes as the delivery vehicle of choice and these are listed in Table 1.

Liposome-Encapsulated Aminoglycosides in Preclinical and Clinical Studies

Aminoglycosides as a class of antibiotics have been successfully used in liposomal preparations and a number of preclinical and clinical studies have been performed (42). Three approaches in particular have been explored, namely, local administration, targeting conventional liposomes to the mononuclear phagocyte system (MPS), and targeting stealth liposomes to locations outside the MPS (42).

Local Application

Liposomes can be considered as a reservoir for prolonged drug release in local application of aminoglycosides. Studies have confirmed this notion when compared with the free drug. One particular study reported promising therapeutic results for the treatment of acquired immunodeficiency syndrome patients with eye infections (43).

Targeting with conventional liposomes. Conventional liposomes are quickly phagocytosed by the MPS and thus exploited for the treatment of intracellular bacterial infections. Studies have reported the prolonged presence of liposomal gentamicin and other aminoglycosides in organs after a single administration (44). Surprisingly, there is a lack of studies examining possible toxicity in humans. However, there are reports comparing acute toxicity of free and liposome-entrapped aminoglycosides in murine models, which show reduced toxicity for the drug-containing liposomes (45,46).

Targeting with stealth liposomes. Long circulating liposomes evade the MPS by providing steric hindrance, which in turn allows for higher concentrations to accrue and more slowly release into the bloodstream. The liposomal drug, amikacin, marketed as MiKasome by NeXstar Pharmaceuticals (Gilead Sciences Inc., San Francisco, CA), consists of a small unilamellar liposomal formulation which was in clinical trials in the mid 1990s (42). Rats receiving 50 mg/kg dose of MiKasome had approximately 130-fold increase of amikacin in the plasma compared with free amikacin. Similar outcomes were seen in

rabbits, dogs, rhesus monkeys, and humans. Increased prolongation of MiKasome were seen in human immunodeficiency virus (HIV) positive patients with no renal toxicity (47,48). For further reading and a thorough review on preclinical and clinical liposomal antibiotics, the reader is referred to Schiffelers and coworkers (42).

Liposomes in Arthritis

Interest in the use of liposomes for the treatment and management of inflammatory arthritis, especially RA, is escalating with a greater emphasis on achieving targeted delivery of liposomes to the synovium (49).

RA is a chronic and progressive autoimmune disorder that is characterized by synovitis and severe joint destruction (50,51). The pathogenesis of RA is complex and involves synovial cell proliferation, cellular immune activation by T, B, and macrophage cells, angiogenesis, pannus formation, and cartilage and bone erosion. The mediators of RA are a network of interdependent systems including cytokines, interleukins (IL-1 and IL-6), tumor necrosis factor (TNFα), prostanoids, proteolytic enzymes, and adhesion molecules such as E-selectin. The aims of treatment are to prevent joint damage, control the inflammatory process, decrease pain, and prevent loss of function. Currently available treatment options include NSAIDs, GCs, disease-modifying antirheumatic drugs (DMARDs), and biological modifying antirheumatic drugs. NSAIDs have analgesic and anti-inflammatory properties but do not alter the course of the disease or prevent joint destruction. GCs, on the other hand, are highly effective for the relief of symptoms and may slow the progression of joint damage. However, these benefits are only temporary due to the adverse drug reactions experienced including osteoporosis, hypertension, weight gain, and fluid retention, resulting in a decrease in long-term use particularly at high doses. The final major group of drugs used in the treatment of RA are DMARDs/biological modifying antirheumatic drugs, which have the potential to reduce or prevent joint damage and function (52,53). Any one of the above drugs or point of disease initiation/propagation has the potential for liposomal targeting.

Nontargeted Liposomes

Liposomal entrapment of prednisolone phosphate. GCs were introduced as therapy for RA in 1950 and were clearly efficacious in the treatment of inflammatory diseases by inhibiting the production of inflammatory cytokines and chemokines. It soon became apparent that prolonged systemic exposure resulted in adverse effects such as osteoporosis, infections, diabetes mellitus, and cataracts (54). Incorporation of GCs into liposomal preparations to minimize the systemic side effects did not go unnoticed. The difficulty faced with liposomal delivery was the lack of specificity in targeting liposomes to sites of inflammation. Strategies examined to overcome this included the following: (a) the use of lipid modified GCs, eg, dexamethasone palmitate that allows greater entrapment in liposomes (55,56); (b) PEG liposomes to reduce nonspecific loss in the RES; (c) passive enclosure of water-soluble GCs by the use of derivatives such as phosphate esters, acetonomide phosphates, and/or succinates in PEG-modified liposomes (57,58); (d) the use of cyclodextrin complexes with GCs (59-61); and (e) new liposomal technologies for the delivery of GCs (62).

In a series of studies performed by Metselaar and coworkers, it was reported that positive effects were observed when liposomal steroids were administered to rats with AIA (63-65). More importantly, the encapsulation of prednisolone phosphate in PEG liposome drastically increased therapeutic efficacy in AIA rats. In one particular study, a single administration of a 10 mg/kg liposomal prednisolone phosphate formulation ameliorated inflammation for almost a week in rats with AIA. On the other hand, the same dose of unencapsulated prednisolone phosphate did not reduce inflammation and only a slight effect was observed following repeated injections (63).

Methotrexate (MTX) Loaded Liposomes

MTX is the most commonly used DMARD for the treatment of inflammatory arthritis. The drug side-effect profile, however, often results in the cessation of therapy. To decrease systemic side effects and optimize the local anti-inflammatory effect, intra-articular free MTX had been administered, but the efficacy was low due to rapid clearance of the drug from the joint cavity (66,67). This can be overcome by encapsulating MTX in liposomes with the aim of increasing retention time in the joint and with the added advantage of targeting macrophages/monocytes in the joints that are propagating the disease.

When tested as a liposomal formulation, MTX leakage was observed (68). To achieve improved uptake, a lipophilic analog of MTX was synthesized by the conjugation of a phospholipid, di-myristoylphosphatidylethanolamine, to the γ-carboxylic acid residue of the glutamyl moiety in MTX. This derivative was significantly better in reducing established joint inflammation than comparable doses of MTX alone (69). Rats treated with liposomal MTX compared with saline-treated animals had less arthritis with no histological changes associated with erosion of bones or cartilage. The authors of the study noted that the beneficial effects observed were due to the inhibition of the local expression of IL-6 and IL-1β mRNA (49).

Superoxide dismutase (SOD) liposomes. One of the characteristics of RA is the overproduction of reactive oxygen species that gives rise to superoxide radicals that irreversibly damage organic compounds and tissues. The possibility of using SOD to catalyze the dismutation of superoxide radicals produced in RA has long been suggested (70). However, the short biological availability of the active substance in SOD and the early severe adverse

reactions noted with the free form of bovine SOD made this form of treatment previously unacceptable (70).

An acylated SOD derivative that has the capability to partially insert in the lipid matrix of liposomes while being partially exposed to the external medium has been constructed (71,72). To improve *in vivo* SOD activity, the enzyme was incorporated either in conventional or in stealth liposomes, which resulted in increased therapeutic activity (71). The liposome-associated enzyme (acylated-SOD-enzymosome) expressed rapid anti-inflammatory effect while circulating in the organism, thus demonstrating that the release of acylated-SOD from the liposome is not required for the expression of enzymatic activity (71). Furthermore, PEG-SOD-containing liposomes have been used for the treatment of arthritis in rats and shown to be therapeutically superior to the "free" enzyme (73,74). Gaspar and coworkers concluded that the enzymosomes combine the advantages of expressing enzymic activity in the intact form as well as sustaining release of the enzyme (75).

Lactoferrin (Lf) -loaded liposomes. The pathogenesis of RA is thought to predominantly involve T-cells and their associated cytokines. Evidence suggests that disruption of the balance between pro-(Th1) and anti-(Th2) inflammatory cytokines is found in RA synovitis (76) and that this balance may be further influenced by the availability of "free" iron in the synovial fluid and tissue. Iron-binding proteins regulate the amount of free iron in the tissue. Lf is an iron-binding glycoprotein from the transferrin family. It binds these potentially toxic-free iron components and thus modulates the inflammatory response (77). Guillen and coworkers have shown that Lf has the ability to bind "free" iron in the synovial fluid of mice with collagen-induced arthritis (CIA) (78). In an effort to further prolong the effect of Lf and modulate the cytokine response of T-lymphocytes, Trif and coworkers administered encapsulated Lf in negatively charged liposomes to DBA/1 CIA mice. Their results showed that Lf liposomes had the ability to prolong tissue residence time and modulate the Th1/Th2 cytokine balance. Treatment with both free and liposomal-entrapped Lf alleviated arthritis compared with control mice. The anti-inflammatory effect was prolonged (2 weeks) in the group treated with Lf-entrapped liposomes compared with free Lf-treated mice (3-4 days) (79).

Clodronate-loaded liposomes. Clodronate is a first-generation bisphosphonate structurally related to an endogenous regulator of calcium metabolism and pyrophosphate (80). Bisphosphonates are resistant to enzymatic hydrolysis and have a long skeletal half-life (81). They are potent inhibitors of bone resorption (82) and have been successfully used for the treatment of hypercalcemia (83). Bisphosphonates in general have antiarthritic effects, which are attributed to their antiresorptive properties and inhibition of macrophage function (84-86).

Tissue destruction by inflammatory cells in arthritis is caused by the persistent recruitment and activation of monocytes/macrophages, which are in abundance in patients with RA. The macrophages in the synovium are capable of ingesting liposomes that localize to the synovium. Incorporation of bisphosphonates into liposomes enables their delivery into macrophages (87). This leads to phagocytic cell apoptosis (88,89), inhibition of metalloproteinases (90), and modulation of macrophage-produced cytokines and nitric oxide (91). Systemically administered clodronate encapsulated in large MLV, stabilized with PEG, suppresses paw inflammation in rats with AIA (92). It was reasoned that these liposomes exerted their effect via a central immunoregulatory mechanism as opposed to the destruction of synovial macrophages (92). By contrast, Richards and coworkers compared the efficacy of a single intravenous injection of either MLV encapsulated clodronate or SUV encapsulated clodronate and noted the latter were more efficient in reducing the severity of inflammation and joint destruction in rat AIA. This effect was associated with the specific elimination of macrophages from the synovial membrane (93). Possibly, clodronate-loaded liposomes remain longer in the circulation while reducing macrophages at the sites of chronic inflammation. This inhibition of histological progression of arthritis supports a central role for synovial macrophages in the progression of inflammation and joint destruction (93). In a different animal model of arthritis, Richards and coworkers showed that the elimination of macrophages induced by SUV led to a reduction in the local production of IL-1β and IL-6, TNFα, and matrix metalloproteinase 9 with resultant temporary anti-inflammatory and anti-erosive effects (94,95).

In a series of experiments by Gomez-Barrena and coworkers (96), it was shown that clodronate-loaded liposomes may be chondroprotective. Bisphosphonates are thought to be chondroprotective in rabbits (97) and humans (98). In response to damaged cartilage, cartilage oligomeric protein (COMP) is released (99). COMP is a fundamental component in maintaining the cartilage matrix and structural integrity by forming bridges between type II and IX collagens (100,101). COMP is a useful serum marker for disease activity and progression of osteoarthritis. In a study by Gomez-Barrena and coworkers (96), low-dose, noncytotoxic liposomal clodronate was administered intra-articularly to antigen-induced arthritic rabbits and COMP levels were assessed. Their results showed that clodronate-loaded liposomes exerted a positive dual effect on synovial membrane (anti-inflammatory) and articular cartilage (chondroprotective). This maintenance of articular cartilage integrity is an important finding with significant clinical implications for the treatment of osteoarthritis.

In an open study, 10 RA patients received a single

intra-articular administration of clodronate liposome. Synovial biopsy following administration showed macrophage depletion and decreased expression of adhesion molecules in the synovial lining. The administration of liposomes was well tolerated (102).

Celecoxib-loaded liposomal gel. The selective inhibitor, celecoxib, used for the treatment of arthritis, was recently reported to have cardiovascular effects and, with the recall of valdecoxib and rofecoxib (103,104), there may be a significant need for research into other modes of drug delivery. Drugs can be delivered to the upper and deeper layers of skin through topical administration, which bypasses gastric degradation and minimizes systemic side effects (105). The major limitation lies in the relatively poor penetration of the drug through the skin. Celecoxib is currently available in 3 forms, namely, tablet, capsule, and suspension (105).

Kaur and coworkers employed a liposomal gel (niosomal) formulation for sustained and targeted (celecoxib) topical delivery to affected joints. Celecoxib-loaded niosome gel provided 6.5 times higher drug deposition (deep skin layer + muscle) compared with the control (carbopol gel). *In vivo* studies revealed significant reduction in rat paw edema when compared with conventional nonliposomal gel (celecoxib), which suggests the liposomal celecoxib gel has the potential for increased skin permeability, localization, and prolonged drug release (106).

Targeted Liposomes

Targeting the monocyte/macrophage system. Autoantibodies and innate immunity mediators secreted by B-cells are important in the K/B×N murine model. Human RA and the K/B×N models have many pathological characteristics in common (107-110). These include the symmetrical involvement of "peripheral" joints in the mice, pannus formation, synovial hyperplasia, and bone and cartilage damage, resulting in the anarchic remodeling of joints. The onset of the disease is dependent on the recognition of an ubiquitous glycolytic enzyme, glucose-6-phosphate isomerase (GPI; residues 282-294) through the KRN T-cell receptor (111). Studies have shown that both the B-cells and the KRN T-cell receptor-expressing cells are necessary to initiate disease (112). The continuation of disease following initiation is due to auto-antigenic anti-GPI antibodies. Transfer of serum obtained from K/B×N mice or affinity-purified anti-GPI antibodies induced arthritis in naive recipient mice (113).

On administration of serum containing GPI-specific autoantibodies, the development of arthritis depended on the presence of mast cells and neutrophils, and the expression of Fc receptor on immune cells (114,115). In support of this, there was no evidence of arthritis when mice were depleted of mast cells and administered K/B×N serum (116). Complete lack of the disease was also noted with the depletion of neutrophils from K/B×N mice (117). Although macrophages play a crucial role in the pathogenesis of arthritis, it is important not to disregard the significant contribution by other inflammatory cell initiators as shown in the above model.

As previously discussed, macrophages play a central role in inflammation and RA due to their production of pro-inflammatory cytokines. At sites of tissue destruction, macrophages are responsible for the production of large amounts of TNF-α, IL-1α, IL-8, prostaglandins, and tissue-degrading proteases (118,119). The degree of macrophages in the inflamed tissue correlates with the severity of the disease in human RA. Therefore, inflammation and tissue destruction is reduced with drugs that can deplete or inactivate macrophages. To study the role of macrophages in the K/B×N mouse model of serum induced arthritis, Solomon and coworkers depleted macrophages from BALB/c mice before the induction of arthritis with the use of clodronate-loaded multilamellar liposomes. Treated mice did not display any pathological signs of the disease on K/B×N serum transfer, suggesting that macrophages play a fundamental role in the pathogenesis of arthritis. To confirm that the absence of macrophages protects mice from arthritis, macrophage reconstitution was performed before the administration of K/B×N serum transfer. Following the depletion of macrophages by clodronate-loaded liposomes, mice were reconstituted with macrophages isolated from healthy mice and then subjected to K/B×N serum transfer; the cohorts then developed arthritis (120).

Targeting strategy involving carbohydrate-loaded liposomes. Carbohydrates are known to play a vital role in cell–cell recognition processes. This property has been used to exploit active targeting capabilities of liposomes (121). In inflammation models such as arthritis, interactions between E-selectin and sialyl Lewis X (SLX) have been examined. The blood group antigen, SLX, is a tetrasaccharide molecule located on the terminus of glycolipids that are present on the surface of white blood cells. Studies have shown that SLX plays an important role in the inflammatory process. The process of adhesion of leukocytes to an inflamed site is mediated by the adhesion molecule, E-selectin, which is specific for SLX. Leukocyte and endothelial cell–cell recognition is thought to occur through lectins and oligosaccharide ligands (122).

Hirai and coworkers have shown that liposomes tagged with SLX home to inflamed tissue. At 24 hours postinjection the SLX liposome complex had shifted from the lining layer of the blood vessels (noted at the 6-hour mark) into the surrounding tissue. Mutual electrostatic repulsion between SLX liposomes and vascular endothelial cells, erythrocytes, and leukocytes was due to the negative charge, which prevents nonspecific attachment. At sites of inflammation, endothelial cell surface characteristics change, allowing for binding of liposomes. Circulation time is extended by decreasing phagocytosis and adsorption of opsonin proteins to the liposome surface (122).

Targeting strategy involving adhesion peptide loaded liposomes. Vascular endothelial cells (VEC) preferentially express growth factor receptors and adhesion molecules for recognition. At sites of inflammation, integrins are up-regulated. To study PEG liposome–vessel wall interactions in living animal models, Koning and coworkers used the mouse dorsal skin flap window chamber model and the AIA rat model (123). Using the dorsal skin flap model, binding of dexamethasone phosphate–containing arginine-glycine-aspartate-PEG-liposomes to the vessel wall of inflamed sites was observed 4 hours post induction of inflammation. This allowed for internalization, which is important for efficient intracellular drug delivery. In the AIA rat model, dexamethasone phosphate-arginine-glycine-aspartate liposomes were efficacious compared with nontargeted liposomal dexamethasone or control vehicle in the treatment of arthritis (123). These results indicate that VECs have an integral role in the inflammatory process and the possibility of using VEC targeting for therapeutic human intervention is a viable option (123).

Targeting strategy involving pH-sensitive liposomes. Construction of liposomes with pH-sensitive polymers/peptides can allow fusion of liposomes with membranes to deliver their contents intracellularly. Recent approaches to intracellular delivery of drugs for DNA and organelle targeting is reviewed by Torchilin (13). The pH (low) insertion peptide (pHLIP) can be used to target acidic tissues found in tumors and inflammatory sites. pHLIP is a 36-amino-acid peptide derived from bacteriorhodospin C helix, which has a number of different physicochemical properties depending on its pH. At physiological pH, the favored conformation allows the peptide to be water-soluble, whereas at acidic pH the changed configuration allows the peptide to insert itself into membranes as α-helix. Finally, at lower pH the peptide can translocate cell-impermeable cargo molecules across cell membranes and release these active compounds in the cytoplasm by reduction of the disulfide bond (124). Fluorescent-labeled pHLIP-containing liposomes were shown to target arthritic knees in AIA models but not normal (control) knees of the same rat. The fluorescence signal was 4 to 5 times greater in the arthritic joint compared with the control (124).

Studies on pHLIPS by Reshetnyak and coworkers led to the conclusion that the mechanism of membrane peptide entry and translocation of molecules into the cells is mediated by the formation of a helix across the lipid bilayer. This was triggered by the increase of peptide hydrophobicity caused by protonation of asparagine residues. Interestingly, Reshetnyak and coworkers also noted the possibility of pHLIPs being used as a new approach for the diagnosis and treatment of various diseases in which a low-pH extracellular environment occurs naturally, eg, tumors, infarcts, inflammation, and infection (125,126).

Targeting strategy involving cell-penetrating proteins/peptides liposomes. Over the last decade, a number of proteins and peptides have been discovered that have the ability to penetrate cell membranes. These include the TAT-protein from the HIV-1, the homeodomain of the Antennapedia transcription factor from *Drosophila* (penetratin), and the herpes simplex virus VP22 transcription factor (127). Within these proteins, small sequences consisting of less than 20 amino acids are generally responsible for penetration across the membrane (127-130). These peptides are collectively referred to as protein transduction domains (PTDs) or cell-penetrating peptides.

Protein transduction was first observed when the full-length HIV TAT protein was discovered to have the ability to enter mammalian cells and activate transcription from an HIV long terminal repeat promotor construct (131,132). The specific region of the protein required for cellular uptake was determined in subsequent studies (133). The mechanism by which PTDs translocate across membranes remains unclear. These peptides have a high proportion of arginine (R) and lysine (K) and it is postulated that these amino acids are essential for plasma membrane translocation by interaction with the negatively charged components of the membrane (134,135).

Nonspecific cellular delivery of antigenic peptides, peptide nucleic acids, antisense oligonucleotides, and full-length proteins, eg, β-galactosidase, can possibly be achieved through PTDs. Furthermore, PTDs can be incorporated into nanoparticles or liposomes. When sufficient amounts of PTDs, such as Antennapedia or TAT, are attached to the liposome, there is significant enhancement of liposome association with cells (130,136-138). Octa-arginine-modified liposome was used to deliver encapsulated plasmid DNA to living cells. The results demonstrate cellular uptake of liposomes coated with these agents occurs via a mechanism independent of vesicular transport (134).

Targeting strategy involving cationic liposome. A novel PEG-coated cationic liposome containing a newly synthesized cationic lipid, 3,5-dipenta-decycloxy-benzamidine hydrochloride (TRX-20), was first reported by Harigai and coworkers as a potential drug delivery system (139). TRX-20 selectively binds to the negatively charged chondroitin sulfate found in subendothelial membranes and certain cell types such as smooth muscle cells, mesangial cells, and synoviocytes (122). The preferential and selective binding characteristics of PEG-coated TRX-20 liposomes to chondroitin sulfate proteoglycans on cell surfaces and in the extracellular matrix of the cells can be a useful tool in drug targeting.

Chondroitin sulfate is highly expressed on the surface of inflamed synovial cells and this has been the target of TRX-20 liposome (139). Rhodamine-labeled prednisolone phosphate-containing TRX-20 liposomes in-

creased the interaction of human fibroblast-like synovial cells by 40-fold when compared with prednisolone phosphate-containing liposomes without TRX-20. These *in vitro* results support the idea that the presence of TRX-20 on the surface of liposomes increases the therapeutic efficacy of GCs in the treatment of RA.

Synovium Targeted Liposomes

Synovial tissue-specific targeting strategies offer the potential to increase drug efficacy while decreasing systemic toxicity and are reviewed by Garrod and Pitzalis (140). Using *in vivo* phage display selection techniques, a number of peptides with homing properties specific for human synovial microvascular endothelium have been identified (141,142).

Other Liposomes in Arthritis

Antiangiogenic Liposomes

To sustain pannus tissue viability and proliferation, new vessel formation (angiogenesis) is required. Often the rheumatoid pannus has been compared with a local invasive malignancy. In principal, the inhibition of angiogenesis will prevent tumor growth or, as in our case, the rheumatoid process. One inhibitor of endothelial cell proliferation is the alkaloid paclitaxel, which has been reported to suppress activity in several tumors. This drug is highly lipophilic and insoluble in water (143,144). Thus the encapsulation of paclitaxel in a liposomal formulation is a logical choice to explore for targeting blood vessels (145).

Liposomal Delivery of Antisense Nucleotides

As biotechnology advances, it may be possible to load liposomes with antisense nucleotides (146), matrix-metalloproteinase-1-specific ribozymes (146-148), and oligonucleotides (149).

Silencing RNA. Administration of TNFα blocking agents such as monoclonal antibodies has beneficial effects in patients with long-term RA and are now commercially available (147). Instead of using neutralizing monoclonal antibodies to TNF, an alternative approach using small interfering RNAs has been successfully used to modulate TNFα expression at both the mRNA and the protein levels *in vitro*. The inhibition was increased with the use of cationic liposomes complexed with TNFα small interfering RNAs and carrier DNA in CIA mouse models following weekly systemic injection of the liposomes (148).

Oligodeoxynucleotides (ODN). DNA-based sequences in the form of ODNs have great potential in the treatment of many disorders, including arthritis, and have been extensively studied in cell culture models. The major hurdle faced in *in vivo* studies has been the exposure of ODN molecules to nucleases in circulation, dilution in the blood, and finally, their binding to other biomolecules (eg, proteins) before reaching their target cell. The use of liposomes to combat difficulties in relation to this issue is on the rise (150).

Successful cellular uptake of ODNs has been achieved with the use of liposomes (anionic, cationic, pH-sensitive, and immunoliposomes). Liposomes composed of cationic lipids prevent nuclease degradation, enhance the rate of ODN cellular uptake, and modify intracellular distribution (151-153). Three vesicular formulations under evaluation for ODN delivery *in vivo* include cationic liposomes, stabilized antisense lipid particles, and hemaglutinating virus of Japan-liposomes (HVJ-liposomes). Although cationic liposomes remain the major form of liposomal delivery of genetic agents, their potential in clinical application can be increased with the reduction of associated toxic effects (150).

HJV-liposomes are also referred to as Sendai-virus liposomes and have been used in the delivery of ODNs *in vivo*. These liposomes are generally composed of lipids in the form of phosphatidylserine, PC, and cholesterol. HVJ-liposomes are encapsulated with the inactivated HVJ (Z strain) virion and are used to deliver transcription factor decoys. Amelioration of arthritis in rat models was seen with direct administration of nuclear factor-kappa B decoy ODNs in HJV-liposomes into the ankle joint (154).

Boron (B) Loaded Liposomes

The B neutron capture therapy in combination with liposomal B delivery offers a novel approach to synovectomy. B neutron capture therapy stems from the ability of ^{10}B nucleus to capture thermal neutrons following radiation. The resultant unstable ^{13}B nucleus undergoes fission to produce a lithium ion and α-particle, as well as radiation (0.48-MeV γ-radiation ($1\ \text{eV} = 1.620 \times 10^{-19}\ \text{J}$)). These energetic fission products share kinetic energy between them for a limited area (approximately 1 cell diameter). The limited range of these products localizes the associated ionization tracking, resulting in cellular damage and subsequent cytotoxicity to those cells that contain a sufficient concentration of ^{10}B. Watson-Clark and coworkers have examined the use of liposomes to deliver B to rats with CIA and noted that this form of therapy may be feasible for the treatment of RA in humans (155).

DISCUSSION

Liposomal-based delivery systems have several limitations that need to be overcome before generalized acceptance. First, liposomes have a limited drug incorporation capacity. Active or remote loading strategies are available for increasing encapsulation efficiency (16,156-158) but overall the efficiency is low (10%). Second, liposomal formulation may inherently have issues with stability due to hydrolysis of ester bonds and oxidation of unsaturated

acyl chains of lipids, for example. Physical instability is caused by possible drug leakage, aggregation of vesicles, and separation of the hydrophobic drug from the bilayer and into the solvent, which ultimately influences the therapeutic index of the drug formulation. Nowadays, there are excellent high-quality products minimizing complications such as hydrolysis, aggregation, fusion, and oxidation. The end result is a greater degree of lyoprotection and subsequent stability (159).

Although the potential of liposomes has not been realized, it has progressed and some commercial preparations are available. For example, liposomes are used for the treatment of fungal infections (amphotericin B), metastatic breast cancer, and Kaposi's sarcoma (doxorubicin, daunorubicin). Their application to arthritis is promising with functional new options being explored, including long-circulating liposomes, conventional liposomes for passive targeting, cationic liposomes for DNA gene transfer, and immunoliposomes for active targeting. Improvements in liposomal formulations and delivery may increase therapeutic activity and improve the benefit–risk ratio, ultimately resulting in liposomal preparations for use in the treatment of arthritis.

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