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# Liposomes in the treatment of inflammatory disorders

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This review focuses on the therapeutic utility of liposomes in the treatment of inflammatory disorders, and aims to offer the reader an overview of the *in vivo* results obtained with liposomally encapsulated anti-inflammatory and immune suppressive drugs. The past 30 years has clearly indicated the added value of liposomes in the search for solutions for the delivery problems encountered. However, only a few liposomal anti-inflammatory therapeutics have entered the clinic. Reasons for the hurdles existing in the translation of promising preclinical findings to clinical studies are discussed.

**Keywords:**inflammatory, disorders, liposomes

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## 1. Introduction

The field of advanced drug delivery liposomes has acquired a front-line position as a drug carrier system. Liposomes are not only the most prominent among the drug carrier systems available, but they are probably also the most widely used for several reasons: liposomes are relatively easy to prepare, can accommodate a broad spectrum of drugs, offer a relatively high drug payload, most liposomes consist of non-toxic and biocompatible lipid constituents, and the liposome surface can be easily modified with specific cell-targeted ligands [1,2].

A particular strength of liposomes is their multi-applicability in the search for adequate solutions to shortcomings and limitations in current drug treatment strategies. They have been used in a wide range of different approaches to improve the performance of a broad range of different therapeutic agents [3].

This review focuses on the therapeutic utility of liposomes in the treatment of inflammatory disorders and aims to offer the reader an overview of the *in vivo* results obtained with liposomally encapsulated anti-inflammatory and immune-suppressive drugs. The perspective from which this review has been written is largely problem-oriented. Over the years a number of investigators have shown the added value of liposomes in the search for solutions for the delivery problems they faced in drug treatment of inflammatory and immune disorders. As this holds true for both topical as well as systemic treatment, the review is organised along the corresponding topical and intravenous routes of administration. Both sections are divided into paragraphs describing the different key functionalities of liposomes that have been shown to be useful to solve problems exhibited by anti-inflammatory and immune suppressive drugs. Although infectious diseases can also involve inflammation, the efforts made to utilise liposomes for the delivery of antibiotics and antifungals is outside the scope of this contribution.

## 2. Topical administration

When the inflammatory disorder in question concerns an organ or tissue that is readily accessible (e.g., skin, eye, lung, joint), and when it manifests itself in one or only a few inflammatory foci (e.g., arthritis in a single joint), topical treatment with anti-inflammatory or immune suppressive drugs may be the first line of treatment.

Besides the advantage of achieving high drug concentrations at the desired target site, another beneficial aspect of topical treatment is that the chance for systemic toxicity of the applied anti-inflammatory and immune suppressive drugs is relatively low. However, there may still be limitations to the successful topical treatment of inflammatory disorders. Drugs can show a lack of retention at the site of application, they can be incapable of reaching their target cell due to an impermeable barrier such as the stratum corneum or cornea. Even when target-site retention and penetration are not an issue, topically administered drugs may show poor therapeutic availability to the actual target cells as a result of premature extracellular degradation and/or insufficient capability to cross the target cell membrane. In addition, although topical administration may be expected to avoid toxicity, drugs may leak away from the site of application and enter healthy surrounding tissues.

Liposomes have been investigated as potential candidates to overcome these limitations. Liposomes can be used to create local depots at the site of inflammation, which improves drug retention; they can be developed into carrier vehicles that take the drug through the impermeable barrier into the target site; they can be used to protect drugs from premature degradation and deliver it in active form to the target cells; and they can protect healthy surrounding tissues against potential harmful toxic effects. These four liposome features offering promise for solving drug-related problems are listed in **Table 1** and are addressed in separate paragraphs below.

### 2.1 Slow-release depot at site of local administration

An important problem associated with local treatment can be limited exposure of the site of inflammation to the applied drug due to rapid disappearance or inactivation. Corticosteroids are a prime example of this problem. They can rapidly pass through biological membranes and are difficult to retain at the site of application.

The therapeutic effects of locally administered liposomal corticosteroid preparations have been studied in experimental arthritis and in dermal inflammation, but mostly in asthma. The earliest work was carried out at the end of the 1970s by Dingle *et al.*, who encapsulated the lipophilic derivative of cortisol, namely cortisol palmitate, into liposomes composed of lecithin and phosphatidic acid, and observed a prolonged anti-inflammatory effect in rabbits with experimental arthritis after intra-articular administration [4,5]. Da Silva *et al.* reported on a rheumatoid arthritis (RA) patient study in the *Lancet* in 1979, in which a beneficial effect was found with intra-articular liposomal cortisol palmitate [6]. During the 1980s more insight of the extent to which liposomes can improve joint retention of corticosteroids was obtained. Bonanomi *et al.* compared liposomal dexamethasone palmitate with unencapsulated microcrystalline triamcinolone acetonide in rabbits with antigen-induced arthritis. Liposomal corticosteroid was more effective and showed better joint retention than unencapsulated corticosteroid. They also showed that the retention could further be improved by increasing the liposome diameter [7]. In the 1990s the experiment was repeated with liposomes containing the palmitate derivative of triamcinolone acetonide in arthritic rabbits by Lopez-Garcia *et al.* The liposomal formulation was more effective than free triamcinolone acetonide. Using radioactive tracers, the investigators found that the liposomal formulation was retained in the inflamed joints for a much longer period of time than free triamcinolone acetonide [8].

Korting *et al.* were the first to evaluate liposomally encapsulated corticosteroids in inflammatory skin disorders. They compared liposomal betamethasone diproprionate with betamethasone gel in patients with atopic eczema and in patients with psoriasis. In this study, only an improvement of therapeutic effect was seen in eczema, but not in psoriasis [9].

However, most studies on local treatment of inflammation with liposomal corticosteroids have concentrated on lung inflammation. Tremblay *et al.* were the first to test liposomal dexamethasone locally in inflamed lungs by using intranasal instillation as the route of administration. In a murine model of hypersensitivity pneumonitis they found an increased activity at the level of bronchoalveolar cells. They observed stronger beneficial effects on histopathology and less systemic activity as compared with free dexamethasone [10]. Dimatteo *et al.* studied the protective effect of intratracheally instilled liposomal dexamethasone on silica-induced pulmonary inflammation in rats. Although they observed protection against silica-induced cellular inflammation and fibrosis, biochemical and functional indices of damage were unaffected or even enhanced [11]. Suntres *et al.* also investigated pulmonary delivery of liposomal dexamethasone. First liposomal dexamethasone was instilled intratracheally in healthy rats. Increased retention in the lung and reduced systemic toxicity was observed with the liposomal drug as compared with free dexamethasone [12]. In a later publication they reported an increased protective effect of intratracheally instilled liposomal dexamethasone versus the free drug in a rat model of lipopolysaccharide-induced lung injury [13]. Waldrep *et al.* were the first to explore the possibilities of pulmonary delivery of liposomal corticosteroids by employing inhalation devices rather than of intratracheal installation. They evaluated the safety of inhaled liposomal beclomethasone diproprionate in healthy volunteers [14]. Improved pulmonary retention of inhaled liposomal beclomethasone diproprionate was later seen by the same group in asthma patients and this observation made the authors speculate about less frequent dosing and consequently better patient compliance in combination with reduced local and systemic side effects [15].

More recent studies focus on the asthma corticosteroid budesonide. Budesonide is known as one of the newer corticosteroids with high local activity and relatively little systemic effects. Joshi *et al.* developed a liposomal budesonide dry powder inhaler yielding prolonged drug levels in the rat lungs [16]. Recently Konduri *et al.* reported increased local retention and duration of the anti-inflammatory activity of aerosolised liposomal budesonide as compared with free budesonide in murine ovalbumin-induced allergic asthma.

**Table 1. Liposome features offering solutions for drug-related problems in topical administration.**

| Liposome feature | Drug-related problem | Example drug | Inflammatory disorders treated | Ref. |
|---|---|---|---|---|
| Slow-release depot at site of local administration | Lack of retention | Corticosteroids | Arthritis<br>Skin disorders<br>Lung inflammation | [4-17] |
| | | Cyclosporin | Allograft rejection | [18,19] |
| Penetration into target site | Poor accessibility of target site from application site | Corticosteroids | Skin disorders | [22,23] |
| | | Diclofenac | Skin disorders | [24] |
| | | Methotrexate | Skin disorders | [26] |
| | | Tacrolimus | Skin disorders | [27] |
| | | Cyclosporin | Skin disorders | [28,29] |
| Extracellular protection or intracellular delivery into target cells | Poor availability in target cells due to extracellular degradation or inability to cross membranes | Clodronate | Arthritis | [31-34] |
| | | Superoxide dismutase | Arthritis | [35-37] |
| | | Lactoferrin | Arthritis | [38] |
| | | Oligonucleotides | Lung inflammation | [41-44] |
| Protection against side effects | Toxicity to healthy nontarget tissue | Dithranol | Skin disorders | [46,47] |

Liposomal budesonide given once a week was demonstrated to reduce inflammation as effectively as free budesonide given once a day [17].

Besides corticosteroids the slow-release attribute of liposomes yielding improved retention in the treatment of inflammatory and immune diseases has only been studied with cyclosporin. Cyclosporin is used as an immune suppressive drug in the treatment of (allo)graft rejection. To treat inflammatory reactions against lung transplants, pulmonary delivered liposomal cyclosporin was considered as this could result in high and retained local concentrations of the drug while leaving healthy nontarget organs less exposed, as compared with the current systemic cyclosporin treatment protocols. Although no therapeutic efficacy data are currently available confirming this hypothesis, the group of Gilbert and Waldrep, who also studied liposomal beclomethasone, reported on two safety and biodistribution studies with aerosolised liposomal cyclosporin. One study concerned the evaluation of the tolerability of a cyclosporin liposome aerosol in healthy volunteers and the other study examined the biodistribution and safety aspects of the same aerosol in dogs. Liposomal cyclosporin was readily and effectively absorbed into the lung epithelium [18,19].

### 2.2 Liposomes as penetration enhancers

In some cases the actual inflammatory foci are located nearby the site of application but are not readily accessible due to the presence of a penetration barrier. A prime example of such a barrier is the top layer of the skin, the statum corneum, which is impermeable for most drugs applied dermally. Several strategies have been employed to open up the stratum corneum for therapeutic agents, such as the use of solvents, the application of electricity and ultrasound [20].

Specific liposome compositions have also been used to enhance penetration of the encapsulated drug through the skin. The most sophisticated liposomal formulation in this respect may be the so-called 'transfersome'. Transfersomes, developed by Cevc *et al.*, are ultradeformable liposomes consisting of phospholipids and a membrane solubiliser such as polysorbate. Transfersomes are reported to overcome the skin barrier by opening extracellular pathways between the cells. As a result of their deformability they are able to enter these pathways and find their way into the subcutaneous tissue [21]. To develop transfersomes that are of use in the treatment of skin inflammation disorders, both steroidal and non-steroidal anti-inflammatory drugs have been encapsulated. In animal models of inflammation Cevc and Blume have been reporting increased and prolonged therapeutic activity of the corticosteroids hydrocortisone, dexamethasone and triamcinolone-acetonide when encapsulated in transfersomes as compared with corticosteroids in conventional creams and ointments [22,23]. In addition, diclofenac was investigated as a non-steroidal anti-inflammatory drug encapsulated in transfersomes in different experimental models of inflammation. Diclofenac associated with transfersomes shows prolonged activity and 10-fold higher concentrations of diclofenac in the subcutaneous tissue in comparison with the free drug in a commercial hydrogel [24].

Elastic, deformable liposomes have also been produced with the help of dipotassium glycyrrhizinate. Trotta *et al.* showed that soy lecithin vesicles in which dipotassium glycyrrhizinate was encapsulated could increase skin penetration of potassium glycyrrhizinate up to fourfold compared with aqueous solutions of this substance [25]. Enhanced skin penetration was also observed with methotrexate encapsulated in dipotassium glycyrrhizinate–lecithin vesicles [26].

Erdogan *et al.* showed that skin penetration can be achieved with less sophisticated liposomal formulations, by achieving therapeutic activity of liposomal tacrolimus in a murine model of psoriasis. In psoriasis, tacrolimus is only effective when systemically administered, but not when topically applied. With the liposomal formulation the authors show a ninefold higher concentration of tacrolimus in the subcutaneous region than after systemic administration of the free drug. The observed therapeutic activity of the topically applied liposomal drug confirms that penetration through the skin into the target site occurs [27].

Verma *et al.* developed a liposomal formulation based on ethanol. Ethanol has been widely reported as an efficient skin-penetration enhancer; however, in principle the solvent is incompatible with lipid vesicles. However, with vesicles composed of a mixture of phosphatidylcholine, lyso-phosphatidylcholine, cephaline, phosphatidic acid and sterol, stable vesicles could be formed in the presence of ~ 25% ethanol. The investigators chose cyclosporin as therapeutic agent. *In vitro*, in human abdominal skin sections, severalfold higher subcutaneous concentrations of encapsulated cyclosporin were found [28]. These findings induced studies on the effect of the cyclosporin lipid vesicles in a rat model of alopecia areata, in which the liposomal cyclosporin containing ethanol and a mixture of penetration enhancers proved to be more effective than liposomal cyclosporin without ethanol as a penetration enhancer. Unencapsulated cyclosporin in ethanol was not effective [29].

### 2.3 Extracellular protection and intracellular delivery into target cells

Despite the aim of topical administration to deliver a maximum quantity of therapeutic agents at the pathological site and limit waste in healthy tissues to a minimum, there are still potential problems that can affect the therapeutic availability of the agent even once it is delivered into the pathological site. These problems include the incapability of the therapeutic agent to cross cell membranes and, therefore, to enter the target cell on its own, or the vulnerability of the therapeutic agent to enzymatic degradation and inactivation. The ideal method of topical administration would, therefore, be to deliver the agent directly inside each single target cell. As this is obviously far from realistic, several therapeutic strategies based on carrier-mediated drug delivery have been developed.

The best example of cell-directed liposomal drug delivery after topical administration is liposomal clodronate. Clodronate, a small drug molecule commonly used against osteoporosis, has been shown to be able to suppress the pro-inflammatory and tissue-damaging activity of macrophages at sites of inflammation. However, free clodronate in solution will not cross cellular phospholipid membranes. In the early 1980s, Van Rooijen *et al.* discovered that liposomally encapsulated clodronate was phagocytosed by macrophages, followed by the intracellular release and accumulation of clodronate. At a certain intracellular concentration, clodronate induces apoptosis of the macrophage [30]. Since then, liposomal clodronate has been widely investigated as a topical treatment strategy in inflammatory disorders, and in particular in arthritis, aiming to suppress inflammation and tissue damage by depleting macrophages from the site of administration [31-34].

Liposomes also offer perspective for therapeutics of biological origin. Several proteins and nucleic acids have shown strong potency *in vitro* but are degraded in biological fluids by proteolytic enzymes and nucleases before being able to reach the actual target cell. The protein that has received most attention by liposomologists for the treatment of inflammatory disorders is the antioxidant enzyme superoxide dismutase (SOD). SOD can neutralise damaging reactive oxygen radicals and has, therefore, received attention as a possible new treatment strategy for RA. The beneficial role liposomes can play in protecting the protein against enzymatic degradation and increasing therapeutic availability at the site of administration is elegantly shown in three papers in which an increased benefit of liposomal SOD over unencapsulated SOD is reported [35-37].

Another protein potentially useful for local therapy of inflammatory disorders is lactoferrin, an iron-binding glycoprotein of the transferrin family that can modulate the inflammatory response by binding harmful iron ions. Trif *et al.* studied a liposomal formulation of lactoferrin in murine collagen-induced arthritis and clearly showed that liposomal encapsulation of lactoferrin results in enhanced retention of intact protein within the injected joint. A positively charged liposome formulation of this protein showed the best results [38].

Besides local delivery of anti-inflammatory proteins, local delivery of antisense oligonucleotides has also been studied to achieve control of inflammation by silencing pro-inflammatory gene expression. However, like proteins, oligonucleotides are rapidly eliminated following local administration and show poor target cell permeation [39,40]. An important point to consider is that not only is degradation in extracellular biological fluids by nucleases a problem, but also intracellular degradation after endosomal and/or lysosomal uptake is a major barrier to overcome for oligonucleotides. For the delivery of nucleic acids, three types of liposomal formulations have been investigated *in vivo* so far. The first being a cationic liposome–oligonucleotide complex, the second a liposome system employing transfecting components of a virus, and the third a liposome that destabilises at low endosomal/lysosomal pH. The first liposome

system is composed of the positively charged dioleoyl trimethylammonium propane and dioleoyl phospatidylethanolamine. Stenton, Ulanova and Befus showed that a complex of this cationic liposome with an antisense oligonucleotide against Syk, a tyrosine kinase that generates pro-inflammatory mediators, can be successfully administered intratracheally in a rat model of allergic pulmonary inflammation. Syk expression in inflammatory cells, the production of pro-inflammatory mediators as well as pulmonary inflammation are effectively reduced by the cationic liposome–oligonucleotide complex [41,42].

The second liposome system is prepared by hydration of a lipid film consisting of phosphatidylserine, phosphatidylcholine and cholesterol with an aqueous solution of the oligonucleotide, and the subsequent mixing of the formed liposomes with purified and UV-inactivated so-called 'haemagglutinating virus of Japan' (HVJ)-liposome. Tomita *et al.* report beneficial effects *in vivo* in a murine model of arthritis with intra-articularly administered HVJ-liposome containing NF-κB decoy oligonucleotide [43], and also evaluated this formulation in RA patients [44]. The production of pro-inflammatory mediators was inhibited and the treatment resulted in a significant inhibition of synovial cell proliferation. The results point to the potential usefulness of properly delivered NF-κB decoy oligonucleotides for local gene therapy of inflammatory disorders.

The third liposomal formulation that has potential for successful intracellular delivery of oligonucleotides into inflammatory target cells is the pH-sensitive liposome system. pH-sensitive liposomes allow oligonucleotides to be released into the cytosol. These vesicles are destabilised by the acidic pH of the endosomes, thus leading to an interaction with the endosomal membrane such that their contents are deposited directly into the cytoplasm. Although their use in inflammatory disorders has been suggested, no *in vivo* data have yet confirmed the feasibility of this concept in the treatment of inflammation [45].

### 2.4 Protection against side effects

Some therapeutic agents effective in the treatment of inflammatory skin disorders pose a threat to healthy tissues. Liposomes have been used to protect the patient against the potential damaging effects of these agents. A prime example is the antipsoriatic drug dithranol. Dithranol (anthralin) has been a consistently effective drug for the treatment of psoriasis for > 80 years but has lost popularity because of the unwanted side effects of irritation and staining. Agarwal *et al.* prepared a gel containing liposomal dithranol and treated patients with psoriasis for 6 weeks in a prospective, open-label trial. In five patients they observed disappearance of the lesions without lesional or perilesional irritation, and without staining of the skin. These preliminary results indicate that liposomal dithranol has potential advantages over presently available preparations of dithranol [46,47]. Other drugs for which topical application is limited by irritation and related effects are benzoyl peroxide and tretinoin, therapeutic agents that have a place in the treatment of acne [48,49].

## 3. Intravenous administration

In many serious inflammatory diseases the target sites are poorly accessible, ensuring that topical treatment is not an option. There can also be a large number of affected body sites, which makes local administration in these cases inconvenient. Examples are RA and related arthritic diseases, multiple sclerosis (MS) and other neurological disorders, and acute or chronic inflammatory diseases related to (allo)graft rejection. These diseases are usually treated by systemic therapy with anti-inflammatory and immune suppressive drugs.

The problems to consider in case of intravenous administration are comparable to those in case of topical administration (**Table 2**). Some drugs show poor pharmacokinetic behaviour (e.g., rapid clearance or a large distribution volume), whereas other drugs are simply too toxic for healthy tissues, thus implying that they are often given at suboptimal doses, which do not yield effective drug concentrations at the target site.

Liposomes can offer a solution to these problems. They can function as a systemic depot after intravenous administration from which the drug is gradually released over time. They can reduce systemic immune suppression by directing the immune suppressive drugs to macrophages of the mononuclear phagocyte system (MPS). Liposomes can extravasate through pathological vasculature and, therefore, selectively accumulate and increase drug concentrations at inflamed target sites, by virtue of the so-called enhanced permeability and retention (EPR) effect [50,51]. Liposomes can also be modified (e.g., by attaching targeting ligands to their surface) such that selective interaction with and uptake by the target cell is achieved. These four critical features of liposomes that can help to overcome the outlined drug-related problems are summarised in **Table 2**.

### 3.1 Slow-release depot in the circulation

Among the earliest investigations with colloidal carriers to improve the pharmacokinetic behaviour of systemically administered anti-inflammatory drugs was the work of Mizushima *et al.* with so-called 'lipid microspheres'. Lipid microspheres are ~ 0.2 μm in size and consist of soy-bean oil and lecithin as the surfactant. These carrier vehicles were shown to be valuable for the improvement of the body distribution behaviour of corticosteroids (liposteroid), prostaglandin E and indomethacin [52-56]. In free form, these therapeutic agents show rapid clearance and poor target accumulation after systemic administration. The authors found a marked increase of therapeutic activity, especially with prostaglandin E encapsulated in lipid microspheres. Monitoring of the body distribution behaviour of radioactively labelled prostaglandin E showed that blood concentrations of prostaglandin E in lipid microspheres were higher and persisted longer than those of free prostaglandin E. These studies with lipid microspheres also revealed the tendency of colloidal carriers to be taken up by macrophages in the liver, spleen and those present in inflamed sites. This MPS uptake phenomenon was later shown to be of value in therapeutic studies with highly toxic immune suppressive agents (see Section 3.2).

**Table 2. Liposome features offering solutions for drug-related problems in intravenous administration.**

| Liposome feature | Drug-related problem | Example drug | Inflammatory disorders treated | Ref. |
|---|---|---|---|---|
| Slow-release depot in the circulation | Poor pharmacokinetics | Corticosteroids | Arthritis and related disorders | [52] |
| | | Indomethacin | Arthritis | [53] |
| | | Prostaglandin | Vascular diseases | [56] |
| Enhanced uptake by macrophages | Systemic immune suppression (toxicity) | Cyclosporin | Allograft rejection | [57-59] |
| | | Tacrolimus | Allograft rejection | [60-62] |
| Extravasation into target tissue | Suboptimal concentration at target site | Methotrexate | Arthritis | [78-81] |
| | | Superoxide dismutase | Arthritis | [82,83] |
| | | Corticosteroids | Arthritis | [84,85] |
| | | | Encephalomyelitis | [86,87] |
| Selective interaction with and uptake by target cells | Inability to enter target cells | Dexamethasone | Hypersensitivity | [96] |
| | | | Arthritis | |

### 3.2 Enhanced uptake by mononuclear phagocyte system macrophages

The first studies with systemically administered anti-inflammatory and immune suppressive drugs encapsulated in liposomes were performed with cyclosporin. Cyclosporin is often used in inflammatory allograft rejection but optimal dosing is limited by nephrotoxicity. Encapsulation in liposomes was shown to reduce nephrotoxicity and to enhance the immune suppressive effect. Mechanistic studies revealed that the increased efficacy is likely to be a consequence of the uptake of the liposomes by macrophages of the MPS in the liver and spleen: cells that play an important role in this type of systemic inflammatory (and immune) disorders [57-59].

Similar results were found following encapsulation of the immune-suppressive agent tacrolimus (FK-506) in liposomes. Increased uptake was found in the liver, spleen and allografts following liposomal encapsulation, whereas localisation and toxicity of tacrolimus in the kidney were substantially reduced [60-62].

### 3.3 Enhanced extravasation and accumulation into inflamed sites

The finding that most liposome types tend to be rapidly taken up from the bloodstream by macrophages in MPS organs is apparently beneficial in the treatment of allograft rejection diseases as previously discussed. However, rapid MPS uptake has since been considered more often as a limitation than a contribution to the treatment of inflammatory and immune disorders. In many inflammatory diseases the inflamed target regions are located outside the MPS system and sufficient extravasation at these non-MPS target sites requires that the liposomes circulate in the blood for a prolonged period of time.

At the end of the 1990s two groups were particularly active in investigating liposome formulations that were instead able to oppose rapid MPS uptake and to localise preferentially in inflamed areas. Love *et al.* showed that accumulation in the inflamed paws of rats with adjuvant arthritis could be increased several-fold by reducing liposome size, modulating liposome charge and increasing the fraction of cholesterol in the lipid bilayer [63,64]. Allen *et al.* achieved the same results by exploring a different approach: they modified the surface of the lipid bilayers first by incorporating the glycolipid ganglioside GM1 and later by coupling the hydrophilic polymer polyethylene glycol (PEG) [65,66]. So far, their strategy has proven most effective in reducing MPS uptake and prolonging the circulation behaviour of liposomes. However, the key finding that triggered worldwide interest in liposome research groups concerns the observation that these so-called 'long-circulating liposomes' (LCLs) spontaneously and selectively accumulate at sites of inflammation, an EPR-based phenomenon often referred to as 'passive targeting' [50,51,67]. In inflamed tissues, the permeability of the vasculature is often increased to the extent that particulate carriers, which are normally excluded from these tissues, can extravasate and localise in the tissue interstitial space, given that they remain in the circulation for a sufficiently long period of time. Accumulation of LCLs into inflamed tissues will create relatively high local drug concentrations, and this obviously opens up an important opportunity to increase the efficacy/safety ratio of many anti-inflammatory and immune-suppressive agents.

Increased vascular permeability is, however, not only observed at inflamed sites but also at sites of infection and in tumours. To date, most research with LCLs has been focused on the field of oncology. Several liposomal formulations are being marketed for the treatment of cancer or are in clinical development. Clinical studies with improved anti-inflammatory therapeutics based on LCLs are still relatively rare, and research is mainly limited to preclinical studies in experimental animal models [3].

Oddly, most of the work with LCLs in inflammatory disorders does not deal with therapeutic agents but imaging agents. LCLs have been successfully labelled with γ-emitting radionuclides such as 99m-technetium and 111-indium and used as a diagnostic tool for the detection of inflammatory and infectious lesions. Both the joint groups of Storm and Corstens, on the one hand, and the group of Phillips and Goins on the other worked on radiolabelled PEG-liposomal formulations. In a range of animal models of infection and inflammation, these groups show that PEG-LCL can indeed be very effective as a radiopharmaceutical for imaging sites of inflammation in the body [68-75]. Two of the more recent publications of the groups of Storm and Corstens report on clinical results with 99m-technetium-labelled PEG-LCL in patients [76,77].

At the end of the 1990s the first studies with anti-inflammatory agents encapsulated in LCLs were performed by the group of Williams. Initially this group investigated methotrexate, a disease-modulating antiarthritic drug, encapsulated in regular liposomes without the long-circulating property. Later, the drug was incorporated in PEG-liposomes to investigate the effect of increased inflamed joint delivery of methotrexate. Remarkably, the investigators did not observe any added benefit of PEG-LCLs over regular liposomes. In their last publication on this issue it was even concluded that LCLs do not appear to have therapeutic potential for treating arthritis [78-81].

However, the value of LCLs for the treatment of arthritis is likely to be dependent on the choice of the encapsulated agent, as has been shown by Storm *et al.* who selected two highly different anti-inflammatory agents for this passive targeting approach. The first papers of the group in this field concerned the increased therapeutic benefit of the protein SOD obtained by encapsulation in PEG-LCLs in rat adjuvant arthritis. The therapeutic potential of SOD-containing PEG-LCLs was established and compared with SOD entrapped in conventional stearylamine-liposomes and unencapsulated (free) SOD. Both PEG-LCLs and stearylamine liposomes showed a superior therapeutic activity compared with SOD in free form, with PEG-LCLs inducing stronger anti-inflammatory effects than stearylamine liposomes [82,83].

The second drug class encapsulated in PEG-LCLs by Storm *et al.* concerned water-soluble corticosteroid derivatives for the treatment of arthritis. Metselaar *et al.* treated rats with adjuvant arthritis and mice with collagen-induced arthritis with a single intravenous injection of PEG-LCLs containing prednisolone phosphate. In both animal models it was observed that LCL-prednisolone phosphate resulted in complete remission of paw inflammation for 1 week, whereas the free drug hardly showed any activity in this dosing schedule. Mechanistic studies showed that the increased therapeutic benefit was a result of selective joint targeting. Besides suppression of the inflammatory response, the process of cartilage erosion could also be significantly retarded [84,85].

Together with Schmidt *et al.*, Storm and colleagues evaluated prednisolone phosphate in PEG-LCL in experimental models of MS. They found that the liposomal drug was strikingly effective at the level of blood–brain barrier disruptions as well as immune cell infiltration into the lesions. Liposomal prednisolone phosphate proved to be superior relative to a fivefold higher dose of free corticosteroid in these experiments [86,87].

In both the experimental arthritis and experimental MS models it was observed that the PEG-LCL, once localised inside the inflammatory lesions, are massively taken up by phagocytic cells. Macrophages at sites of inflammation are known to play a crucial role in the disease by producing pro-inflammatory mediators and tissue-damaging enzymes. The observation that macrophages at inflammatory sites can be targeted with LCLs suggests the opportunity to modulate or even deplete inflammatory macrophages at inflamed sites. Indeed, beneficial effects were seen with LCLs containing clodronate, an agent that can cause apoptosis in macrophages following phagocytosis of the liposomal form of this drug. Richards *et al.* observed in rat models of antigen-induced arthritis and streptococcal cell wall arthritis that a single injection of clodronate encapsulated in small LCLs resulted in selective depletion of synovial macrophages, with a beneficial effect at the level of disease activity as a consequence [88,89].

### 3.4 Selective interaction with and uptake by target cells

Active targeting of liposomes refers to the conjugation of targeting ligands to the surface of liposomes to obtain specific binding to cell receptors on the surface of the target cells. In the field of inflammatory disorders, active targeting with liposomes may be used to realise two different aims. First, one can think of selective targeting of endothelial cells at inflamed sites by employing liposomes targeted to specific inflammation markers expressed on their surface, such as adhesion molecules [90]. Second, selective interaction with target cells after the liposome system has entered the inflamed sites may be pursued. In the latter case active targeting aims to improve the therapeutic availability of liposomal drugs to target cells present within the inflamed site and to minimise undesired side effects on nontarget cells within the same sites [91]. For successful ligand-targeted therapeutics both the choice of the targeting ligand and the cell-surface epitope to which the ligand is directed are critically important.

Although many groups have been investigating the concept of active targeting using liposomes, most of the *in vivo* work so far is confined to the field of tumour targeting. Regarding the first aim, to target inflamed endothelium, an important step forward was made *in vivo* by employing the finding that cationic liposomes can selectively interact with angiogenic endothelial cells. Thurston *et al.* fluorescently labelled liposomes composed of positively charged dioleoyl trimethylammonium propane or dimethyldioactadecyl ammonium bromide and cholesterol, and observed in a

murine chronic airway inflammation model preferential localisation of intravenously administered cationic liposomes at the angiogenic blood vessels in the inflamed sites. Further mechanistic studies revealed that these cationic liposomes were mostly localised inside the endothelial cells, and that only a small fraction extravasated through the vessel walls as in the case of passive targeting [92].

Targeting endothelial cells by exploiting cell-specific surface markers has been widely investigated *in vitro*. Liposomes have been modified with ligands that can selectively interact with E-selectin, ICAM-1 and $\alpha_v\beta_3$, molecules that are upregulated on the surface of endothelial cells following activation by inflammatory signals [93-95]. The only *in vivo* work has been performed by Everts *et al.* (with E-selectin-targeted liposomes) and Koning *et al.* (with $\alpha_v\beta_3$-targeted liposomes). The biodistribution and target localisation of E-selectin-targeted dexamethasone-containing liposomes modified with anti-E-selectin antibody was examined in a murine delayed-type hypersensitivity model. Enhanced uptake by activated endothelium at inflamed sites as compared with control tissue was observed [96]. Koning *et al.* studied liposomes targeted against $\alpha_v\beta_3$ at sites of inflammation *in vivo*. Dexamethasone was encapsulated as active ingredient, and besides selective endothelial cell binding, a long-lasting antiarthritic effect in the rat model of adjuvant arthritis was observed.

The second aim, to accomplish selective interaction with target cells after extravasation of the targeted liposomes into the inflamed regions, has hardly been addressed *in vivo* as yet. *In vitro* work suggests that selective targeting and modulation of lymphocytes that play a role in antigen recognition and activation of the immune system may be a promising approach in arthritis [97]. Others propose selective targeting of dendritic cells and other antigen-presenting cells that play a crucial role in antigen presentation. The only *in vivo* work so far in inflammatory disorders has been performed by Boot *et al.*, who showed that the surface receptor CD134, specifically expressed by auto-aggressive T cells at sites of inflammation, can efficiently be targeted by liposomes modified with anti-CD134 antibody. It was found that encapsulation of 5′-fluorodeoxyuridine dipalmitate in these liposomes could lead to inactivation of autoaggressive T cells and amelioration of experimental arthritis [98].

## 4. Expert opinion

The development of liposomal therapeutics for the treatment of inflammatory and immune disorders has somewhat lagged behind the development of oncological liposomal formulations. For the purpose of cancer therapy several liposomal products are already available on the market or in early-to-late stage clinical trials. This does not mean, however, that the application of liposomes for the treatment of anti-inflammatory disorders is less promising. In fact, with 100 publications cited herein dealing with *in vivo* work with liposomes, this review shows that liposomes make up arrears in the field of inflammatory and immune diseases.

However, no liposomal anti-inflammatory therapeutics have reached the market yet and relatively little are in the stage of clinical development. Regarding topical treatment, only transfersomes [99], antipsoriatic dithranol liposomes [46] and intra-articular clodronate liposomes for the treatment of RA [33] have entered the clinic. Regarding systemic treatment, it is mainly the work by Mizushima *et al.* on lipid microspheres that has reached the clinic [100,101].

Apparently, hurdles exist in the translation of promising preclinical findings to clinical studies. One hurdle concerns the recent development of biological therapies that specifically target key pro-inflammatory cytokines (e.g., the TNF inhibitors etanercept, infliximab and adalimumab). This means that other advanced and targeted therapies such as liposomal anti-inflammatory therapeutics increasingly face competition in the market. The potential benefit of liposomal encapsulation must, therefore, clearly outweigh the costs.

Another more obvious hurdle is the high cost associated with clinical trials. Clinical development, therefore, requires the involvement of the pharmaceutical industry, and this leads to the real bottleneck because the industry remains cautious regarding the development of advanced drug delivery systems.

As investment in the clinical development of new therapeutics is driven by the anticipated commercial value, the extent to which the technology behind the product can be protected by patents is a major concern. Many potential new liposomal therapeutics presented in this review are based on technologies that have already been patented and published. First, this may seriously jeopardise the issues of novelty and inventiveness, which are required to get a patent granted. Second, even if these requirements are sufficiently met and the patent is granted, there is still a high risk of infringement into patents owned by other parties.

To improve the chance that promising liposomal therapeutics can actually be developed into new products for the treatment of inflammatory and immune diseases, it is crucial for academic groups to consider patentability of their work at an early stage by sufficiently addressing issues of novelty and inventiveness. Besides this, it is essential that companies that own patented liposome technologies on which new liposomal therapeutics are based are willing to obtain or to grant licenses to enable further industrial development.

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