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REVIEW

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Liposomal Drug Formulations in the Treatment of Rheumatoid Arthritis

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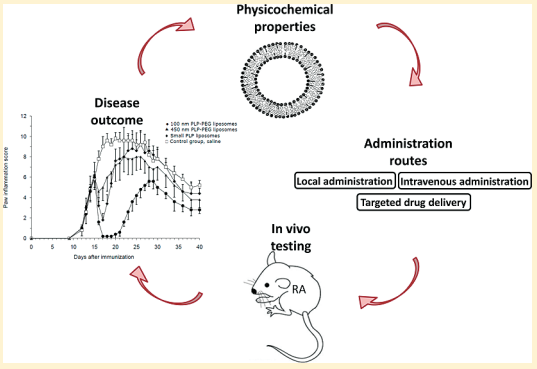
ABSTRACT: Liposomes have been extensively investigated as drug delivery systems in the treatment of rheumatoid arthritis (RA). Low bioavailability, high clearance rates and limited selectivity of several important drugs used for RA treatment require high and frequent dosing to achieve sufficient therapeutic efficacy. However, high doses also increase the risk for systemic side effects. The use of liposomes as drug carriers may increase the therapeutic index of these antirheumatic drugs. Liposomal physicochemical properties can be changed to optimize penetration through biological barriers and retention at the site of administration, and to prevent premature degradation and toxicity to nontarget tissues. Optimal liposomal properties depend on the administration route: large-sized liposomes show good retention upon local injection, small-sized liposomes are better suited to achieve passive targeting. PEGylation reduces the uptake of the liposomes by liver and spleen, and increases the circulation time, resulting in increased localization at the inflamed site due to the enhanced permeability and retention (EPR) effect. Additionally liposomal surfaces can be modified to achieve selective delivery of the encapsulated drug to specific target cells in RA. This review gives an overview of liposomal drug formulations studied in a preclinical setting as well as in clinical practice. It covers the use of liposomes for existing antirheumatic drugs as well as for new possible treatment strategies for RA. Both local administration of liposomal depot formulations and intravenous administration of passively and actively targeted liposomes are reviewed.



KEYWORDS: liposomes, rheumatoid arthritis, antirheumatic drugs, targeted drug delivery

1. INTRODUCTION

RA is a systemic inflammatory disease characterized by chronic, progressive inflammation and gradual joint destruction. The primary target of the inflammatory process is the synovial tissue. Activated macrophages produce inflammatory cytokines that cause ongoing inflammation, joint swelling, bone erosion and cartilage damage. This results in pain, swelling, stiffness and functional impairment.[1−4] Currently, there is no cure for RA.[5] The goal of treatment is 2-fold: to alleviate the burden on the patient and to minimize joint damage.[3,4] Because of low bioavailability, high clearance rates and limited selectivity of several important drugs used for RA treatment, high and frequent dosing is often required to reach satisfying therapeutic effects. However, such intensive treatment also increases the risk for the occurrence of severe side effects.[2,6]

Liposomes have been investigated extensively as drug delivery vehicles to increase the therapeutic index of the encapsulated drug, and their versatility to accommodate a wide range of therapeutic agents has been demonstrated in preclinical and clinical settings.[7] Liposomal physicochemical properties can be changed to optimize passage of biological barriers and retention at the target site, and to prevent premature degradation and toxicity to nontarget tissues.[8−14] Over the years liposomes have proven to be well tolerated carrier vehicles, as most liposomes consist of (semi)natural, biodegradable lipids.[6] Despite these advantages, only a few liposomal products have entered the market, with, as leading examples, Doxil (or Caelyx, in Europe), Myocet (liposomal doxorubicin), and Ambisome (liposomal amphotericin B).

Liposomal formulations can be applied locally as well as systemically. Local administration can be applied when the disorder is localized to only a single or a limited number of sites and when the site of pathology concerns a tissue that is readily accessible, as can be the case in RA. After systemic administration, the liposomal carrier system has to deliver the drug to the site of action. To achieve this, the so-called "passive targeting" phenomenon can be

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employed. Inflamed tissues are characterized by enhanced vascular permeability, which allows small, long-circulating drug carrier systems to extravasate at these sites via the EPR effect. Subsequently they are retained in the extravascular space (often referred to as the EPR effect),[15,16] with a large portion being taken up by macrophages in the synovial layer.[17,18] Passive targeting and the EPR effect make the use of long-circulating liposomes attractive for improving the therapeutic index of antirheumatic drugs. Furthermore, by coupling of targeting structures to the liposomal membrane, specific cell populations can be targeted in the pathological site (also referred to as "active targeting"). This strategy can potentially further improve the selectivity of the formulation.

This review provides an overview of liposomal drug formulations studied for use in the treatment of rheumatoid arthritis. Both local and systemic administration routes are addressed.

2. RHEUMATOID ARTHRITIS

2.1. Clinical Symptoms. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease, often polyarticular, affecting multiple smaller and larger joints throughout the body. The prevalence is about 1%, and women are 3 times more prone to develop RA than men.[1,2] Although considered an autoimmune disorder, the exact cause is unknown.

The primary target of the inflammatory process is the synovial tissue. Inflammation of the synovial tissue is characterized by formation of an edematous and highly vascularized "pannus-like" tissue that progressively invades and degrades underlying articular cartilage and bone.[19] This pannus tissue originates from the synovial lining and consists of synovial macrophages, synovial fibroblasts and infiltrating inflammatory cells such as activated T and B lymphocytes. In addition to the invading pannus, the contents and volume of the synovial fluid are affected. Digestive enzymes (e.g., matrix metalloproteinases, MMPs) are secreted and attack surrounding tissue. Additionally, oxidative stress caused by reactive oxygen species (ROS) secreted by activated macrophages and other blood-derived cells is thought to contribute to tissue destruction.[20] Joint and tissue destruction are the hallmarks of RA that ultimately culminate in immobility and deformity.

2.2. Current Treatment Strategies. Available treatment options for RA aim at symptomatic pain relief with nonsteroidal anti-inflammatory drugs (NSAIDs) on the one hand, and slowing down disease activity and aiming for remission with disease modifying antirheumatic drugs (DMARDs) and corticosteroids on the other hand.[2,3]

NSAIDs are drugs with analgesic, antipyretic and anti-inflammatory effects. Most NSAIDs act as nonselective inhibitors of the enzyme cyclooxygenase (COX-1 and COX-2), which catalyzes the formation of prostaglandins from arachidonic acid. Prostaglandins act as messenger molecules in the process of inflammation. Many NSAIDs display a short half-life after oral administration, demanding frequent and high dosing to achieve a full therapeutic effect in RA, thus increasing the risk for gastrointestinal side effects.[1,2]

DMARDs are effective in slowing down disease progression. The mechanism of action of most classic (synthetic) DMARDs in RA is still unclear. The antimetabolite methotrexate (MTX) is considered to be the most important and useful DMARD.[21,22] It has an acceptable toxicity profile at low doses and can be given orally. Other frequently used classic DMARDs are sulfasalazine, hydroxychloroquine, leflunomide, cyclosporin, intramuscular gold injections and azathioprine. More recently, biological DMARDs, such as tumor necrosis factor-alpha (TNFα) blockers and interleukin-1 (IL-1) blockers, have been developed.[5,23] These drugs appear to be highly effective as single agents as well as in combination with other DMARDs.[24] Quite a few new biological DMARDs have been developed, and some are close to entering the market.

Glucocorticoids (GCs) are useful both as temporary therapy, until the response to DMARDs is achieved, and as chronic therapy in severe RA that is not well controlled with use of DMARDs. GCs are a class of steroid hormones with well-known immunosuppressive and anti-inflammatory effects, primarily as a result of their ability to modulate DNA transcription through binding to the cytosolic glucocorticoid receptor.[25−28] At higher concentrations, GCs can also induce nongenomic anti-inflammatory and immunosuppressive effects.[29] The use of GCs is hampered by their highly unfavorable pharmacokinetic properties, i.e., rapid clearance and a large volume of distribution, which necessitates high and frequent dosing to maintain therapeutic levels at sites of inflammation, which increases the risk for severe adverse effects, especially upon long-term treatment.[26,30,31]

Currently, international recommendations for the treatment of RA are not available. Therefore, the European League Against Rheumatism (EULAR) aims to develop standards for this treatment. Based on five systematic literature reviews on available treatment options and related economic issues,[22,24,28,32,33] three overarching principles and 15 recommendations were made, which are summarized in the treatment diagram in Figure 1.[34,35] Nowadays, treatment of RA often starts with the use of NSAIDs.[2−4] However, since cartilage damage and bone erosions are known to occur already at early disease states, the EULAR recommends an early start with DMARD treatment, skipping NSAID treatment.[32] If possible a patient should start on MTX; otherwise leflunomide, sulfasalazine or injectable gold could be considered.[22,34,35] If a patient response to the therapy is insufficient, the treatment should be adapted as soon as possible (within 1−3 months). First, a change to another synthetic DMARD or, if prognostically poor factors are present, addition of a biological DMARD (especially a TNF-inhibitor) should be considered. If the first TNF-inhibitor fails, a second one can be tried. GCs can be used as initial, short-term treatment, but their use should be tapered as soon as possible.[22,24,28,32−35] It was shown that this treatment strategy was cost-effective.[33] When the disease is stable for at least 12 months, slowly tapering off the biological and subsequently the synthetic DMARDs could be considered. However, discontinuation of DMARD therapy is associated with increased flare frequency, and moreover, remission is much harder to achieve after discontinuation of DMARD therapy. Therefore, tapering DMARDs should be performed cautiously and should be monitored strictly.[34]

3. LOCAL ADMINISTRATION

In some cases RA is restricted to only one or a few larger joints (commonly referred to as monoarthritis or oligoarthritis, respectively), which provides an opportunity for local treatment via the intra-articular (ia) route, resulting in a high local concentration with potentially minimal systemic exposure. The benefits of local treatment, however, can be limited by poor retention of the therapeutic agent in the joint.[6,36,37] To improve and prolong drug exposure of the inflamed area after a single ia injection, liposomes have been studied as drug depot formulations after ia administration. To minimize systemic exposure, clearance of the drug from the joint after release from the liposome should be low.

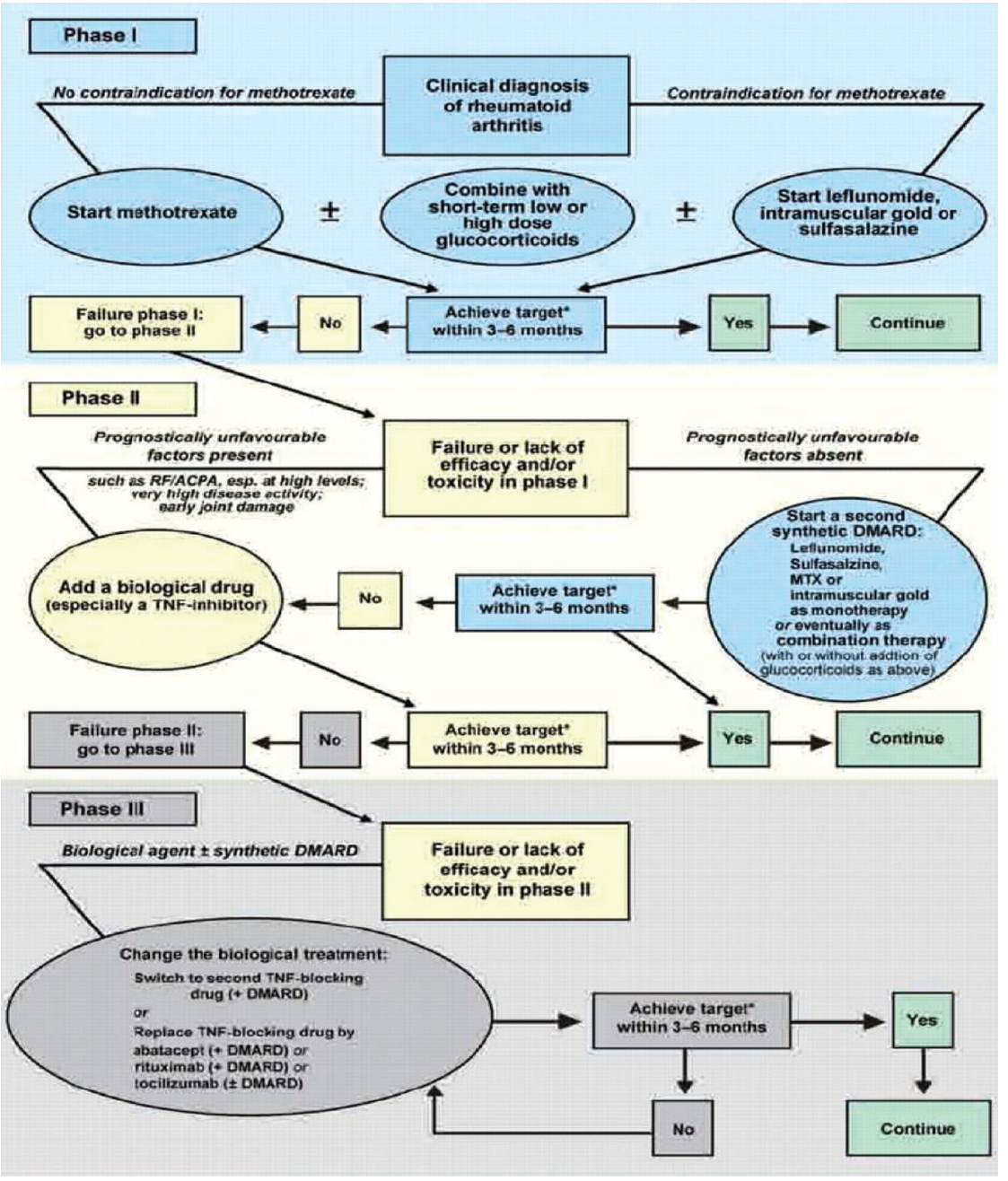

Figure 1. Treatment strategy based on the European League Against Rheumatism recommendations on rheumatoid arthritis management. DMARD, disease-modifying antirheumatic drug; MTX, methotrexate; RF/ACPA, rheumatoid factor/anticitrullinated peptide antibodies; TNF, tumor necrosis factor. *The treatment target is clinical remission or, if remission is unlikely to be achievable, at least low disease activity. Reprinted with permission from EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Smolen, J. S. et al. *Ann. Rheum. Dis.* **2010**, *69*, 964−975. Copyright 2010 BMJ Publishing Group Limited.

3.1. Methotrexate. Already in 1988, Foong et al. tested an ia formulation of liposomal MTX (Table 1: A) in a rabbit model for arthritis. The clearance of free MTX from the joint is very fast after ia administration.[38−40] To reduce this clearance, MTX was encapsulated in the aqueous interior of the liposomes. A 40-fold increase in drug retention in the joint was found, compared to injection of free MTX. However, only 4% of the liposomal MTX was associated with the synovium. Liposomal MTX was approximately 10-fold more effective in suppressing the development of arthritis compared to free MTX, when injected at the time of disease induction. Neither free nor liposomal MTX was effective in the suppression of synovitis in established arthritis.[40,41] Williams et al. state that this is because of rapid leakage of MTX from the liposomes, followed by rapid clearance from the joint. Therefore, they covalently coupled MTX to the phospholipid DMPE and incorporated this lipophilic derivative in the phospholipid bilayer of large multilamellar (MLV) and small unilamellar vesicles (SUV) (Table 1: B and C).[42] Fast leakage of the drug was successfully minimized by using the lipophilic derivative MTX-DMPE. A single ia injection of the MLV formulation in rats

Table 1. Liposomal Drug Formulations in the Treatment of RA

label	type	drug	∅ (nm)	composition (molar ratio)	route of administration	animal model	ref
A	not defined	MTX	1070	EPC/CHOL/DCP (5:5:1)	ia	rabbit AIA	40,41
B	MLV	MTX-DMPE	1200	EL/CHOL/PA (7:2:1)	i.a	rat AIA	42
C	SUV	MTX-DMPE	100	EL/CHOL/PA (7:2:1)	ia, iv	rat AIA	42,105
D	MLV	MTX-DMPE	1,200	POPC/CHOL/DMGP (7:2:1)	ia	rat AIA	43
E	not defined	DFNa	235 or 242	DMPC or DSPC/CHOL/DCP (7:1:2)	ia	rabbit AIA	45,46
F	niosomes	DFNa	270	SUR I/CHOL/DCP (7:1:2)	ia	rabbit AIA	
G	lipogelosomes	DFNa	235 or 242	DMPC or DSPC/CHOL/DCP (7:1:2) mixed 1:1 (w:w) with 1% C-940 or CMC-Na	ia	rabbit AIA	
H	niogelosomes	DFNa	270	SUR I/CHOL/DCP (7:1:2) mixed 1:1 (w:w) with 1% C-940 or CMC-Na	ia	rabbit AIA	
I	HA-BAL (MLV)	DEX, DFNa	unknown	SPC/DPPE (95:5)+HA	ia	monosodium iodoacetate model of OA in rats	47
J	COL-BAL (MLV)	DEX, DFNa	unknown	SPC/DPPE (95:5)+COL	ia	monosodium iodoacetate model of OA in rats	
K	not defined	TAC	unknown	DPPC/CHOL/PA (8:3:1)	ia	carrageenan induced paw edema model in rats	13
L	not defined	DMP	160	EPC/octyl glucoside mixtures (10:51.2)	ia	rabbit AIA	8
M	OLV	DMP	4500	EPC/octyl glucoside mixtures (10:51.2)	ia	rabbit AIA	9
N	OLV	DMP	750	EPC/PA/C8E4 (10:1:56.2)	ia	rabbit AIA	8,9
O	MLV	DMP	950	EPC/SA/C8E4 (10:1:56.2)	ia	rabbit AIA	9
P	MLV	DMP	2000	EPC/PA (10:1)	ia	rabbit AIA	
Q	MLV	DMP	9000	DPPC/PA (10:1)	ia	rabbit AIA	
R	not defined	clodronate	1000	PC/CHOL (∼10:1 w/w)	ia	healthy mice, mouse CIA	50,51
S	not defined	clodronate	120−160	PC/CHOL (unknown ratio)	ia	RA patients (human)	52
T	not defined	clodronate	unknown	DSPG/DSPC/CHOL (unknown ratio)	ia	rabbit AIA	53
U	liposomes(+)	lactoferrin	200	PC/CHOL/PS (5:5:1)	ia	mouse CIA	57,58
V	liposomes(+)	lactoferrin	200	DOPE/CHEMS (6:4)	ia	mouse CIA	
W	liposomes(−)	lactoferrin	200	DPPE/CHOL/SA (5:5:1)	ia	mouse CIA	
X	LUV	APO2L/TRAIL	unknown	EPC/SM/CHOL/DOGS-NTA (unknown ratio)	ia	rabbit AIA	61
Y	liposomes(−)	SOD	110	EPC/DSPE-PEG/CHOL/SA (unknown ratio)	sc	rat AA	63
Z	liposomes(−)	SOD	450	EPC/DSPE-PEG/CHOL/SA (unknown ratio)	sc	rat AA	
a	transferrosomes	SOD	150	SPC/sodium cholate (3.75:1)	ec	rat AA	64,65
b	PEG-liposomes	MTX-DMPE	100	DSPC/CHOL/DSPE-PEG (10:5:1)	iv	healthy rats, rat CIA	69,70
c	LUV	indomethacin	100	EPC/CHOL/SA (1:0.5:0.1)	ip	carrageenan induced paw edema model in rats	71
d	PEG-liposomes	PLP, DXP, BUP	100/450	DPPC/CHOL/DSPE-PEG2000 (1.85:1.0:0.15)	iv	rat AA, rat CIA	17,72,76
e	non-PEG-liposomes	DXP	285−310	DPPC/DPPG/CHOL (50:10:40 mol %)	iv	rat AIA, rat CIA	73−75
f	liposomes	PLP	100	DPPC/CHOL (2.0:1.0)	iv	rat AA	76
g	PHEA-liposomes	PLP	150	DPPC/CHOL/PHEA-lipid-conjugate (1.85:1.0:0.15)	iv	rat AA	78
h	PEG-liposomes	MPHS, BMHS	85	HSPC/CHOL/DSPE-PEG2000 (55:40:5)	iv	rat AIA, healthy beagle dog	79
i	MLV	clodronate	unknown, large-sized	PEG-S/SDS/CHOL (4:1:4)	iv	rat AA	83
j	MLV	clodronate	unknown, large-sized	CHOL/PC/PS (40:80:8 w/w/w)	iv	sheep AIA	84
k	not defined	clodronate	100	EPC/CHOL/DPPA (7:7:1)	iv	rat AIA, SCW induced arthritis model in rats	86,87
m	MLV	clodronate	unknown, larger-sized	EPC/CHOL (2:1)	iv	rat AIA	86
n	liposomes(+)	SOD	200/100	EPC/CHOL/SA (7:2:1)	iv	rat AA	88,89,91
o	PEG-liposomes	SOD	110/200	EPC/CHOL/DSPE-PEG2000 (1.85:1.0:0.15)	iv	rat AA	
p	not defined		65	DSPC/CHOL/lipophillic boron species (3:3:1)	iv	rat CIA	93
q	not defined		88	DSPC/CHOL/lipophillic boron species (3:3:2)	iv	rat CIA	
r	not defined		65	DSPC/CHOL/hydrophilic boron species (3:3:1)	iv	rat CIA	

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Table 1. Continued

label	type	drug	∅ (nm)	composition (molar ratio)	route of administration	animal model	ref
s	not defined		88	DSPC/CHOL/hydrophilic boron species (3:3:2)	iv	rat CIA	
t	liposomes(+)	siRNA targeting TNFα, IL-1, IL-6 and/or IL-18	2000−3000	DOPE/cationic lipid RPR209120/DNA-carrier (unknown ratio)	iv	mouse CIA	96,97
u	not defined		100	DPPC/CHOL/DCP/ganglioside/DPPE/sodium cholate (16.8:10.1:1.8:14.6:2.3:46.9 w/w/w/w/w/w) mixed 1:1 with DTSSP, +SLX (up to various densities)	iv	mAb induced arthritis in mice	99
v	PEG-liposomes	DXP	100	DPPC/CHOL/DSPE-PEG2000/DSPE-PEG2000-maleimide (1.85:1.0:0.075:0.075)	iv	rat AIA	100
w	PEG-liposomes	FUdR-dP	100−200	EPC/CHOL/DSPE-PEG2000/DSPE-PEG2000-maleimide (2:1:0.075:0.075)	sc	rat AA	103

with experimental arthritis resulted in a rapid and sustained anti-inflammatory effect, superior to the effect of the SUV formulation. This was explained by the fact that the larger liposomes were more effectively retained in the inflamed joint compared to the smaller liposomes. Further experiments showed that a single ia injection of a comparable formulation of MLVs (Table 1: D) was able to reduce knee joint swelling to values comparable to nonarthritic knees in rats already after 7 days.[43] It was not investigated if the biological activity of MTX was changed due to the chemical coupling to DMPE. Further research is needed to elucidate this possible change in biological activity.

3.2. NSAIDs. The NSAID diclofenac sodium (DFNa) has attracted increasing attention as a valuable agent in the treatment of RA, due to the quick onset of analgesic effects and anti-inflammatory properties. However, DFNa has a very short plasma half-life and can evoke adverse gastrointestinal side effects.[44] Therefore, Türker et al. prepared various drug delivery systems for local administration of DFNa to the inflamed joints, to avoid systemic exposure and increase the local exposure time. Both liposomes and niosomes (i.e., vesicles prepared from nonionic surfactants) of approximately 250 nm were compared to formulations that involve injectable hydrogels in which these vesicles were incorporated (lipogelosomes and niogelosomes, respectively)[45,46] (Table 1: E−H). 49−67% of the radiolabeled carrier was still present in the arthritic joint of rabbits 24 h after ia injection of these formulations.[45] The retention in the joint improved with increased viscosity of the formulation. Additionally, the release of DFNa in the most optimal formulation (Table 1: G using DPMC and C-940) is determined not only by release from the liposomes but also by release of free drug from the surrounding gel network. Treatment with this DFNa-loaded lipogelosome formulation reduced joint swelling 90% compared to the unaffected joint in arthritic rabbits. Cartilage damage and bone erosion were prevented.[46]

DFNa was also encapsulated in bioadhesive liposomes (BAL), carrying hyaluronan (HA) or collagen (COL) on their surface (Table 1: I and J). These liposomes have a high affinity for specific sites and molecules in the target area such as extracellular matrix, integrins, cartilage components and hyaluronan receptors, resulting in an increased retention of the liposomes in the joint. In rat osteoarthritis, a reduction of the inflammation of the knee joint over a time span of 17 days was seen after treatment with both types of liposomes. The most effective treatment was generated by combining DFNa and dexamethasone (DEX) in HA-BAL, which yielded a reduction of the knee inflammation to 12.9% of its initial volume, as was calculated from the MRI data. No reduction in body weight was seen, pointing to acceptable tolerability of the formulation.[47]

3.3. Glucocorticoids. To increase the retention of GCs in the joint cavity, Lopez-Garcia et al. compared an ia injection of liposomal triamcinolone acetonide 21-palmitate (TAC-P) (Table 1: K) to ia injections of free triamcinolone acetonide (TAC) in rabbit arthritis. Due to the palmitate anchor, the drug is expected to be incorporated in the liposomal membrane. Whether the palmitate anchor has an effect on the biological activity of TAC has not been reported. The liposomal formulation induced increased retention in the articular cavity: 8 h after treatment 38% of the liposomal TAC-P was still present in the joint cavity, while the free TAC was already completely cleared from the joint cavity within 1 h. This retention correlates with the increased reduction in paw diameter observed.[13] Similar results were reported by Elron-Gross et al. for BAL containing DEX.[47]

Bonanomi et al. entrapped the fatty acid-derivatized GC dexamethasone palmitate (DMP) in liposomes of different sizes (100 nm up to 30 μm), lamellarity, charge and lipid composition (Table 1: L−Q), to improve the stability in the joint after ia administration.[8,9] The retention of the various types of liposomes was compared to that of unencapsulated microcrystalline suspensions, containing both dexamethasone phosphate (DXP) and TAC, after a single ia injection in rabbits with arthritis. The retention in the synovium of healthy rabbits was optimal for liposomes with a mean diameter of more than 750 nm, with 6 times more intact large DMP-liposomes present in synovial fluid as compared to small DMP-liposomes 48 h after ia injection.[8] The small DMP-liposomes showed a three times better anti-inflammatory response after 24 h compared to DXP/TAC-suspension in a three-times higher dose. The therapeutic effect of the large DMP-liposomes was not tested. None of the liposomal formulations suppressed the endogenous plasma cortisol.

3.4. Other Therapeutic Agents. *3.4.1. Clodronate.* Macrophages play a key role in RA, mainly by excreting a range of potent pro-inflammatory mediators and enzymes.[48,49] Besides, they are responsible for clearance of liposomes by the mononuclear phagocyte system (MPS). Therefore, macrophages can be considered an interesting target cell population for liposomal drugs. Clodronate (dichloromethylene bisphosphonate) is a drug that induces apoptosis when delivered intracellularly into

macrophages. Depletion of macrophages in the synovium by liposomal clodronate has been pursued experimentally to decrease the inflammation.[49]

Van Lent et al. encapsulated clodronate in liposomes, for selective delivery of the drug to joint macrophages. This highly water-soluble drug cannot cross cell membranes in its free form and was therefore encapsulated in liposomes (Table 1: R), to achieve intracellular delivery in macrophages. After ia administration, clodronate was intracellularly released from the liposomes and induced apoptosis.[50,51] Prophylactic depletion of local macrophages before induction of arthritis completely blocked immune cell infiltration and onset of arthritis in mice, showing the importance of macrophages in the initiation and maintenance of chronic arthritis.[50] Barrera et al. studied this approach in RA patients and showed that macrophages were successfully depleted using liposomal clodronate (Table 1: S) and that this procedure was well-tolerated.[52] Thus far, this is the only clinical study reported in the literature that uses liposomal drug formulations.

Čeponis et al. demonstrated that weekly ia injections of low doses of liposomal clodronate (Table 1: T) had anti-inflammatory and joint-sparing effects in arthritic rabbits, without being cytotoxic for cells. Significantly less TNFα was found in the synovium of liposomal clodronate-treated rabbits, as compared to untreated rabbits. However, the effect was only temporary and it did not prevent the occurrence of joint erosions over the long term.[53] Besides induction of apoptosis, low, noncytotoxic ia doses of liposomal clodronate appear to have chondroprotective and anti-inflammatory effects on damaged cartilage by the enhancement of levels of cartilage oligomeric protein (COMP), an integral structure component of the cartilage matrix, as was shown in a rabbit model for arthritis by Gomez-Barrena et al. This means that liposomal clodronate can also have an important function in the repair potential of the cartilage, as it helps to strengthen the collagen network.[54]

3.4.2. Lactoferrin. Liposomes have also been employed for the effective retention of macromolecular drugs in arthritic joints after ia administration. In RA, iron can potentially act as a catalyst in the production of damaging free radicals. Endogenous iron-binding proteins are often unable to bind all the iron that accumulates in synovial tissue and fluid. The enzyme lactoferrin (Lf) is a glycoprotein that can bind free iron.[55] Guillén et al. showed that periarticular injection (i.e., around the joint) of Lf significantly suppressed the inflammation. However, 75% of the injected Lf was cleared from the infected joint within 6 h and the anti-inflammatory effect lasted only for 3 days.[56] Therefore, Trif et al. entrapped Lf in liposomes (Table 1: U−W) and compared the retention of ^{125}I-labeled liposomal Lf to the free protein after a single ia injection in arthritic mice.[57,58] Free Lf was poorly retained in the joint, with 62% of the initial dose lost 2 h postinjection and only 2% remaining at 24 h (Figure 2). Entrapment in positively charged liposomes of 200 nm strongly increased the retention, with close to 50% of the initial dose still present at 6 h and 15% at 24 h postinjection (Figure 2). After a single ia injection of the positively charged liposome formulation, the arthritis severity decreased continuously over the full observation period of 12 days. Additionally, this liposomal Lf formulation reduced the pro-inflammatory cytokine production and increased the anti-inflammatory cytokine production compared, to free Lf.[58] Entrapment in negatively charged liposomes did not improve the joint retention, and after 24 h the Lf had already completely disappeared from the arthritic joint.[57]

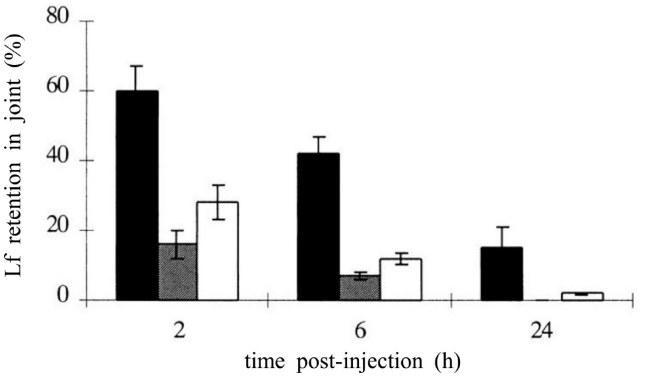

Figure 2. Joint retention (% of injected dose) of ^{125}I-labeled Lf after a single ia injection in arthritic mice. One milligram of ^{125}I-labeled Lf was administered as free protein (Lf (free)) or encapsulated in 200 nm charged liposomes (either in Lf-L (positive) or in Lf-L (negative)). Adapted from ref 57. Copyright 2001 Society for Experimental Biology and Medicine.

3.4.3. Biologicals. Recently a number of biological agents, the majority of which block TNFα, have been developed. One of these compounds is APO2L/TRAIL, which consists of 2 proteins related to the TNF family, which both induce apotosis.[59] Ia injection of APO2L/TRAIL leads to apoptosis of synovial cells that contribute to joint destruction. However, the compound needs to associate with exosomes in the synovial fluid for its biological activity, and it was shown that exosome levels are extremely low in RA patients.[60] Therefore Martinez-Lostao et al. conjugated APO2L/TRAIL to liposomes (Table 1: X). The liposomes can take over the function of the exosomes, mimicking the natural active form of APO2L/TRAIL. These APO2L/TRAIL liposomes were injected in the inflamed joint space in a mouse model, and this resulted in a reduction of synovial hyperplasia to almost normal values and 60% less joint inflammation, compared to only 30% for nonliposomal recombinant APO2L/TRAIL.[61] Further studies regarding the effectiveness of this liposomal protein formulation are still ongoing.

It has been proposed that the antioxidant enzyme superoxide dismutase (SOD) protects cells from radical oxygen species (ROS), by catalyzing the dismutation of the toxic superoxide radical anion to oxygen and hydrogen peroxide.[20] However, a major limitation of the therapeutic use of SOD is its short half-life of about 6 min after iv administration.[62] Therefore, Simões et al. and Corvo et al. used liposomal formulations for local administration of SOD. Corvo et al. investigated the SOD delivery to the inflamed joint after subcutaneous injection (sc) of small-sized (110 nm, Table 1: Y) and larger-sized (450 nm, Table 1: Z) PEGylated liposomes in arthritic rats. The large-sized liposomes were retained at the site of injection to a 2-fold higher extent compared to the small-size liposomes. The uptake in the inflamed joint was 17-fold higher for the small-sized liposomes compared to large-sized liposomes. Sc administration of small-sized liposomes appeared to be as effective as iv administration, suggesting that the small-sized liposomes reach the circulation and are targeted to the inflamed area by the EPR effect.[63]

Simões et al. focused on a novel route of administration: carrier-mediated transdermal transport with transferosomes (Tfs, Table 1: a). Tfs are ultradeformable mixed lipid liposomes, specifically developed for transdermal delivery of compounds.[64] Tfs with a mean particle diameter of 150 nm were loaded with

SOD and applied epicutaneously (ec) on bare skin of arthritic rats.[65] Daily ec application of SOD-Tfs (0.66 and 1.0 mg/kg body weight) appeared to have a larger anti-inflammatory effect compared to daily iv administration of long-circulating SOD-PEG-liposomes (0.066 mg/kg body weight). This paper provided for the first time evidence that transport of intact and therapeutically active enzymes from the healthy outer skin to the systemic circulation by entrapment in Tfs is possible.

4. INTRAVENOUS ADMINISTRATION, PASSIVE TARGETING

When the target joint is not accessible for local administration, the drug may be targeted to the inflamed area after systemic administration. Inflamed tissues allow small, long-circulating drug carrier systems to extravasate by the EPR effect, referred to as passive targeting.[15,16] Traditional liposomes have a short blood circulation time after intravenous (iv) administration, due to rapid and efficient uptake by macrophages of the MPS, mainly those in the liver and spleen. Although targeting macrophages in liver and spleen might have a positive effect reducing the splenomegaly that is often seen in RA patients, rapid uptake of drug loaded liposomes by the MPS is not the prime consideration for using a liposomal drug formulation. For optimal use of the EPR effect to reach the target organ, i.e., the inflamed synovium, stable and long circulating liposomes are necessary. Long-circulating liposome formulations have been prepared by modifying the surface of liposomes with hydrophilic polymers such as poly(ethylene glycol) (PEG)[2,66] or, more recently, poly(vinyl alcohol) (PVA)[67] and poly(amino acids) (PAA),[68] all coatings which can effectively oppose uptake by macrophages of the MPS.

4.1. Methotrexate. Williams et al. encapsulated MTX-DMPE in the bilayers of 100 nm non-PEGylated liposomes (Table 1: C) and in 100 nm long-circulating PEG-liposomes (Table 1: b) and compared their therapeutic efficacy and toxicity in an arthritis model in rats.[43,69,70] It was anticipated that the long-circulating PEG-liposomes would accumulate in the inflamed joints, thereby delivering more MTX to the target tissues compared to the non-PEGylated ones. Surprisingly, the non-PEGylated liposomes showed considerable anti-inflammatory potency while the long-circulating PEG-liposomes did not reduce joint-swelling as compared to the saline-treated control group.[69,70] Further testing showed that the non-PEGylated liposomes were more rapidly cleared from the circulation and taken up in the inflamed joint compared to the PEG-liposomes (1.5 h vs 24 h). This is reflected by the onset of the effect: joint swelling decreased already after 2 consecutive daily injections of non-PEGylated liposomes, while for the long-circulating PEG-liposomes this effect was started not earlier than day 6 after initiation of treatment.[69] These findings were unexpected. The authors hypothesized that delivery to the macrophages is less effective for the PEG-coated liposomes, resulting in a later onset of the effect.[70] So in the short term, non-PEGylated liposomes seem to reach the target more efficiently, but in the long term, the long-circulating ones are as efficient.[69] The toxicity of liposomal MTX was reduced when compared to free MTX,[70] indicating that the free MTX levels in plasma after liposomal administration are quite low. No significant changes in red blood cell counts were observed after 4 days of treatment, but white blood cells and platelet counts were significantly lowered.

4.2. NSAIDs. Despite their proven therapeutic value, the high incidence of (gastrointestinal) side effects limits the use of NSAIDs in RA. Therefore, various groups have developed liposomes for local administration.[8,9,13,47] For systemic administration, targeting the drug to the inflamed joints, however, only attempts for indomethacin have been made thus far.

Srinath et al. developed and optimized a liposomal formulation for indomethacin (Table 1: c). The lipophilic drug is incorporated in the lipid membrane, and its acid moiety has an electrostatic interaction with the amine moiety of the lipids in the membrane. As a result of this interaction, release from the liposome is quite slow. The liposomal formulation was significantly more effective in the inhibition of edema volume in rat models for arthritis, while the size and severity of occurring ulcers were reduced compared to administration of free indomethacin.[71] Further studies regarding the efficacy and safety of this formulation are ongoing.

4.3. Glucocorticoids. The potential of long-circulating liposomes to target GCs to sites of inflammation after iv administration, increasing their therapeutic index, was proven in several preclinical studies. Several GCs were tested, among which were prednisolone disodium phosphate (PLP), dexamethasone disodium phosphate (DXP) (Table 1: d,[72] e[73−75]) and budesonide disodium phosphate (BUP) (Table 1: d[72]). PLP was encapsulated in 100 nm long-circulating PEG-liposomes (Table 1: d) and tested in rat and mouse models of arthritis.[17,76] A single iv dose of 10 mg/kg of free PLP did not result in a significant effect on paw inflammation, while the same single dose of PEGylated PLP liposomes resulted in the complete disappearance of the clinical signs of arthritis within 2−5 days (Figure 3). The anti-inflammatory effect lasted for one week, after which joint inflammation gradually reappeared. Daily iv injections of the same doses of free PLP for 5−7 consecutive days and single injections of the same dose of PLP encapsulated in small 100 nm non-PEG liposomes (Table 1: f) and large PEG-liposomes (450 nm diameter) were all much less effective (Figure 3). Both latter liposomal formulations showed enhanced uptake by macrophages in the liver and spleen and diminished accumulation in inflamed paws.

Given the fact that PEG is not easily degraded, it cannot be excluded that PEG accumulates intracellularly where it could interfere with cellular processes.[77] Therefore biodegradable alternatives to PEG have been developed, like poly(hydroxyethyl L-asparagine) (PHEA),[78] PVA[67] and PAA.[68] The therapeutic activity of PLP encapsulated in biodegradable PHEA-liposomes (Table 1: g) in arthritic rats was equal to that of PLP encapsulated in PEG-liposomes at the same dose.[78]

Compared to liposomal PLP, liposomal DXP had a comparable anti-inflammatory effect at a five times lower dose (2 mg/kg DXP vs 10 mg/kg PLP) in arthritic rats.[72] Liposomal treatment with DXP could reduce the dose of DXP by a factor of 3−10 compared to free DXP.[74,75]

Treatment with BUP in PEG-liposomes at a dose of 1 mg/kg was as effective as DXP in PEG-liposomes at a dose of 2 mg/kg in arthritic rats, while showing hardly any systemic adverse events. Therefore BUP might be a promising candidate for liposomal encapsulation.[72]

Avnir et al. hypothesized that a higher encapsulation efficiency and a higher molar drug to lipid ratio might provide a formulation with superior overall characteristics.[79] Amphipathic weak acid prodrugs methylprednisolone hemisuccinate (MPHS) or betamethasone hemisuccinate (BMHS) were loaded into 85 nm PEG-liposomes using a remote loading technique described by Clerc et al.[80] The pharmacokinetics and the anti-inflammatory effect of these remote loaded liposomes (Table 1: h) were compared to

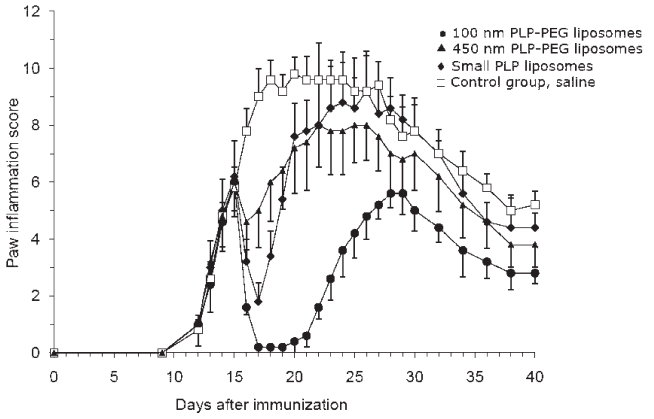

Figure 3. Therapeutic effect of a single iv injection of 10 mg/kg PLP encapsulated in long-circulating PEG-liposomes (■ 100 nm PLP-PEG-L), large PEG-liposomes (▲ 450 nm PLP-PEG-L) and 100 nm non-PEGylated liposomes (● PLP-L) as compared to treatment with saline as a control (□) in arthritic rats. Arrow indicates treatment day. Adapted with permission from ref 76. Copyright 2003 American College of Rheumatology.

free MPHS and BMHS in rats with arthritis and in a beagle dog.[79] The pharmacokinetic results obtained in both animal species were very similar. Treatment with both liposomal formulations resulted in complete remission of inflammation three days after the first injection, after which joint swelling increased within two weeks to values comparable to those of control animals.[79]

4.4. Other Therapeutic Strategies Using Passive Targeting of Liposomes. *4.4.1. Clodronate.* As discussed above, bisphosphonates can be used for depletion of local macrophages to reduce the inflammation in RA. Initial experiments with iv administration of liposomal formulations focused on the influence of liposome size and composition. After iv injection, small liposomes (in the 100 nm range) were shown to accumulate to a significantly greater extent than large liposomes (in the μm range) in inflamed joints.[81] Larger-sized clodronate liposomes reduced joint swelling, but did not prevent joint destruction.[82,83] This result was confirmed by an experiment by Kinne et al., who showed that multilamellar clodronate-containing vesicles (Table 1: i) induced depletion of macrophages in the liver and spleen, but had no effect on the macrophages in the synovial layer, while a significant reduction in joint swelling was seen.[83] Highton et al. tested large-sized clodronate liposomes (Table 1: j) in a sheep model for arthritis. In this model no reduction of joint swelling was seen, although they did show that the liposomes reached the inflamed synovium.[84]

To more specifically target the macrophages in the inflamed joint, Love et al. developed small-sized clodronate liposomes. Depletion of macrophages in the joint would reduce the inflammation.[85] Richards et al. tested these clodronate-containing small-sized liposomes (Table 1: k) in arthritic rats for their ability to deplete synovial macrophages, and compared them to larger multilamellar liposomes containing clodronate (Table 1: m) after iv injection.[86] A single iv dose of small-sized liposomes was more effective than larger-sized ones, sustaining a significant reduction in knee swelling for up to 7 days. The limited efficacy of the larger-sized liposomes was attributed to its strong localization in the MPS, where it effectively depleted the hepatosplenic macrophages. Large liposomes thus failed to accumulate in inflamed joints after systemic administration.[86] Richards et al. later assessed the prophylactic effect of liposomal clodronate on the onset of arthritis when treated 10 days after disease induction.[87] The local macrophage elimination that resulted from administration of small-sized clodronate liposomes significantly suppressed the development of arthritis and induced a significant reduction in synovial levels of proinflammatory interleukins, TNF-α and MMPs.[87]

4.4.2. Superoxide Dismutase. As already addressed, SOD can be used to protect cells against ROS, but it has a plasma half-life of about 6 min. To avoid rapid clearance via the kidneys, Corvo et al. encapsulated SOD in 3 types of radiolabeled liposomes (Table 1: n and o).[88] Additionally, they strongly improved the encapsulation efficiency of SOD into PEG-liposomes (Table 1: o) by transiently lowering the pH to 3.3 (below the isoelectric point of the protein), yielding a positively charged protein that showed enhanced interaction with the negatively charged lipids during the formation of the liposomes. Readjusting the pH to 5.6 fully restored the enzymatic activity of SOD.[89] These PEGylated liposomes were subsequently compared for their therapeutic activity to non-PEGylated liposomes, after iv administration to rats with arthritis, in a dose range of 33 to 363 μg/rat.[89] The PEGylated liposomes resulted in 3-fold (200 nm) and 8-fold (100 nm) higher blood levels than the positively charged stearylamine-containing liposomes, 24 h after administration.[89] The prolonged circulation time and small size resulted in a strong accumulation in the inflamed areas. However, even repeated daily administration of the optimal formulation during the course of disease development was not able to completely eliminate joint swelling.[63]

Gaspar et al. state that this limited therapeutic efficacy of liposomal SOD could be caused by a limitation in the extent and/or rate of release in the inflamed area. Therefore, they developed a new formulation of SOD, in which they covalently conjugated multiple fatty acid chains to SOD, rendering a more lipophilic acylated SOD, with only a 10% reduction of the enzymatic activity.[90] When incorporated in PEG-liposomes, it retained its enzymatic activity, while being partly present at the liposomal surface. This can be considered an advantage, because release is not longer needed for therapeutic activity.[90] The therapeutic potential of these so-called enzymosomes (liposomes containing surface-presented SOD, Table 1: n and o) were compared with PEGylated and non-PEGylated (SA containing) liposomes containing conventional SOD within their interior at doses of 33, 165, and 363 μg per animal in arthritic rats (Table 1: n and o).[91] As expected, the circulation time of the PEGylated liposomes was longer than for the non-PEGylated ones, reflected also by a lower hepatosplenic uptake. The therapeutic benefit of enzymosomes presenting modified SOD on their surface was a faster onset of anti-inflammatory activity after iv injection, indeed suggesting that surface-exposed SOD exerted its enzymatic effect without the need to release the encapsulated SOD.[91]

4.4.3. Therapeutic Strategies Using Activation after Injection. In photodynamic therapy a photosensitizing drug is delivered to its site of action where it is activated using a selective wavelength of laser light. Upon activation, the photosensitizing agent forms short-lived oxygen derived species that induce damage and anoxia and promote apoptosis of the affected cells. Chowdhary et al. treated arthritic rabbits iv with the photosensitizing drug BPD-verteporfin (liposomal BPD-MA) to determine the time and dose dependency for reduction of joint inflammation. BPD-MA was delivered by the liposomes to the inflamed synovium as well as the surrounding tissues with a high degree of vascularization, but was also rapidly cleared from the synovium. It was concluded

that, to treat the synovium, early light exposure is needed. Apoptosis was seen in 27% of the cells in the synovium, making targeted photodynamic therapy a possible treatment strategy in RA.[92]

Another strategy tested in RA is boron neutron capture therapy (BNCT). BNCT is a form of radiotherapy that depends on the interaction of slow neutrons, applied by a neutron beam, with ^{10}B that was injected to the patient. Upon absorption of a neutron, the heavier ^{11}B disintegrates into a lithium nucleus (^{7}Li) and an α particle, without producing other types of ionizing radiation. These particles cause ionizations over a length of only one cell diameter, thereby sparing the surrounding tissues. Watson-Clark et al. targeted the boron to the inflamed synovium using liposomes. For this type of therapy, at least 15 μg of boron per gram of target tissue is needed. Liposomal formulations containing different amounts of boron species embedded in the vesicle bilayer (Table 1: p and q) or encapsulated in the aqueous core (Table 1: r and s) were compared. The liposomes were administered iv in a rat model for arthritis. Boron was delivered to the synovium and retained there, resulting in a final boron concentration of 26 μg per g of tissue, and a synovium/blood ratio of 2.0 after 48 h, after which the boron level slowly decreases to 14 μg per g of tissue after 96 h (Table 1: s). Further research is needed to test the efficacy and safety of this approach.[93] However, during irradiation, a high number of neutrons have to be directed to the target. Currently, only nuclear reactors are able to provide such a neutron beam and the energy spectra of the neutron beams available can differ considerably, which limits the clinical application of BNCT. For a multicenter clinical trial to be conducted the beam energy has to be standardized, which currently seems very difficult to achieve.[94,95]

4.4.4. Small Interfering RNA (siRNA). In living cells RNA interference is a naturally occurring mechanism to control the expression of genes. This mechanism is exploited by delivery of siRNA into target cells, to stop the production of a certain protein. In RA, TNFα is one of the most prominent cytokines, and by silencing TNFα transcription in macrophages, the disease activity can be reduced. To specifically deliver siRNA designed to silence TNFα to the macrophages in the inflamed joints, a liposomal formulation was developed by Khoury et al. (Table 1: t). Complete cure was seen in arthritic mice after iv treatment with 10 μg of siRNA encapsulated in cationic liposomes. Due to their large size (2−3 μm) a large part of the liposomes was targeted to liver and spleen, but despite this, the inflamed joints were also targeted. The TNFα secretion was decreased by 50−70%, resulting in a reduction of the incidence and severity of the inflammation.[96] Besides TNFα, Khoury et al. also encapsulated siRNA designed to silence IL-1, IL-6 and IL-18 (Table 1: t). Iv administration of the liposomes resulted in a delay of the onset of the disease, a reduction of the incidence and severity of the inflammation and an inhibition of proinflammatory gene expression (both local and systemic). The best results were obtained when the three different siRNA sequences were combined in the same liposome, attacking different pathways of disease development. This combination was as effective in reducing paw swelling and arthritis severity in mice as the liposomal formulation containing siRNA against TNFα.[97]

5. INTRAVENOUS ADMINISTRATION, ACTIVE TARGETING

Targeting ligands can be coupled to the liposomal surface to enable binding to receptors (over)expressed at the target site, referred to as active targeting. In RA, macrophages are an obvious target, but as described above, these cells can be targeted using passive targeting mechanisms. Besides passive targeting, several actively targeted liposomal formulations have been designed to target other cell types that play important roles in RA: endothelial cells and T-cells.

5.1. Active Targeting to Vascular Endothelial Cells (VECs). VECs at the inflamed site play a crucial role in inflammatory processes. At the same time, they provide easy access to iv administered drug carrier formulations. Therefore, VECs are an interesting target for the treatment of RA.

The cell adhesion molecule E-selectin is a suitable target molecule because it is selectively expressed on VECs activated by cytokines at sites of inflammation.[98,99] To target E-selectin, the tetrasaccharide sialyl-Lewis X (SLX), the natural ligand for E-selectin, was conjugated to the surface of 100 nm liposomes, containing a fluorescent substance (Table 1: u). The accumulation of SLX-liposomes in sites of inflammation in arthritic mice was compared to liposomes lacking the ligand or bearing an irrelevant ligand. SLX-liposomes accumulated in sites of inflammation to a greater extent compared to control liposomes, as was visualized using scanning fluorescent microscopy. It is thought that this selective accumulation occurs via the same pathway as the accumulation of leucocytes in inflamed areas, since leucocytes also express SLX on their surface. The anti-inflammatory effect of drugs loaded into these liposomes has not yet been investigated.[99]

Another way to target VECs is by exploiting the strong upregulation of the integrin αvβ3 on angiogenic VECs at sites of inflammation.[100] Cyclic Arg-Gly-Asp sequence-containing peptides (cRGD) have been developed as specific high-affinity ligands for these αvβ3 integrins.[101] Koning et al. encapsulated DXP in 100 nm PEG-liposomes, with cyclic RGD peptides covalently attached to the distal ends of the PEG chains (Table 1: v).[100] After iv administration to rats at the onset of arthritis, the RGD-PEG-liposomes were cleared more rapidly from the circulation compared to the control PEG-liposomes. However, 3-fold higher accumulation at sites of inflammation compared to the control PEG-liposomes was achieved. This suggests that the specific targeting mechanism is more effective than the EPR effect used in passive targeting in reaching the target site in an early state of the arthritis.[100] A single iv injection of DXP loaded into these RGD-PEG-liposomes had a strong and prolonged anti-inflammatory effect in rats with experimental arthritis, which was by far more efficacious than DXP loaded into passively targeting PEG-liposomes. This indicates that active targeting to VECs at the inflamed site might be a favorable way to treat RA.[100]

5.2. Active Targeting of Autoaggressive T-Cells. It is hypothesized that, besides macrophages, autoreactive T-cells play a major role in the etiology of RA. Autoreactive T-cells secrete cytokines that activate synovial macrophages and fibroblasts, and thereby contribute to the inflammatory process. By blocking these T-cells selectively, the production of cytokines can be reduced.[102] Upon activation, $CD4^+$T-cells in RA express the activation marker CD134. These autoaggressive $CD4^+$T cells are mainly present in the synovial fluid in RA patients. To selectively block these T-cells, Boot et al. actively targeted activated autoaggressive $CD4^+$ T-cells that show upregulation of the expression of surface marker CD134.[103] PEG-liposomes were coated with monoclonal antibodies against CD134 (Table 1: w) and were tested in a rat model for arthritis. Although the anti-CD134-liposomes were shown to specifically bind to the activated T-cells, they were not internalized. This unexpected finding led to the use of a fatty-acid-derivatized drug to enable lipid-coupled

drug transfer between the liposomal membrane and the cell membrane of the target T-cell to achieve intracellular drug delivery. In this study dipalmitate-5′-fluorodeoxyuridine (FudR-dP) was used. Indeed, the severity of the developing arthritis was reduced, albeit only to a moderate extent.[103]

6. CONCLUDING REMARKS

Extensive attention has been given to the concept of liposomes as drug carriers to improve the therapeutic index of drugs. However, with respect to the application of liposomal drug delivery in the treatment of RA, the literature is still limited and fragmentary, and lacks systematic and comparative studies. Nevertheless, as clearly evidenced by the literature reviewed in this contribution, liposomal carriers can be very functional to improve the therapeutic performance of anti-inflammatory agents in RA, either by introducing a depot (local administration) or by attaining site specific drug targeting (intravenous administration). It is obvious that liposomes compete with other delivery systems in this field, but they are particularly attractive by virtue of their great flexibility in terms of composition, physicochemical characteristics and ability to accommodate a wide spectrum of drug molecules. Large-sized liposomes are particularly attractive to achieve slow release effects upon local administration. When administered locally, liposomal drugs have been demonstrated to be more effectively retained compared to the free drug. Local treatment with a liposomal formulation could be indicated when the disease is limited to only a few, readily accessible joints. Small-sized liposomes are better suited to achieve targeting after intravenous administration. Surface modifications can be introduced to further improve target localization by prolonging the circulation time (passive targeting) and/or by interacting with specific target cell receptors (active targeting).

Overviewing the preclinical literature, particularly liposomal formulations of methotrexate and glucocorticoids appear to be promising candidates for further translational studies into their role as therapeutic intervention when exacerbations occur. In the case of liposomal methotrexate, the advantage would be that the patient does not have to switch to a therapy with another drug molecule. In the current recommendations, the use of GC in the treatment of RA is to be kept as low as possible.[28,34] However, the publications on liposomal GCs reviewed here suggest that liposomal formulations could change this point of view.

Traditional therapies in RA have consisted of anti-inflammatory and immunomodulatory agents, and both therapeutic classes may exert undesirable side effects. NSAIDs, systemic glucocorticoids and methotrexate or other DMARDs are known to cause renal, gastrointestinal, neurologic, hematologic or immunologic toxicities. Also with the newer biological therapeutics, there is a need to improve their side effect profiles. The use of liposomes represents an attractive strategy to overcome toxicity problems associated with these traditional and newer therapeutic agents.

Currently, many promising new therapeutic agents are entering the market or are in the late phase of clinical studies. Most of these are biologicals, specifically binding to proinflammatory cytokines and other proteins, like infliximab, etanercept, cetolizumab pegol, golimumab and adalimumab (all anti-TNFα), tocilizumab (anti-IL-6), anakinra (anti-IL-1), abatacept (anti-CD28) and rituximab (anti-CD20). However, despite their success, the most important issue with the biologicals is the price, though the costs will likely decrease over time.[2,33,34] Also a range of new small-molecular agents are in development, such as the kinase inhibitors INCB-28050, tasocitinib and fostamatinib disodium, which are currently in phase II and III trials.[104]

With all these successful new therapies lining up, an important question to address here is whether or not there will still be a market for novel liposomal formulations in RA. While this review shows that liposomes can be highly functional in the target therapy of RA, no liposomal formulations for the treatment of RA have been marketed yet, nor are there any in clinical development at this moment. To date, only one clinical study had been published.[52] Apparently, the translation of the reported preclinical successes into clinical application is not straightforward. This may partly be explained by the fact that industry prefers novel therapeutic products and new chemical entities over improved liposomal reformulations of existing (often generic) therapeutic agents. Also, in relation to the generic compound in its free form, the market price of its improved liposomal equivalent is likely going to be several times higher, and it is questionable whether or not the potential improvement of the therapeutic index by the liposomal formulation is going to be sufficiently valuable from a clinical perspective to allow for such a price premium. Most of the current literature demonstrates improved efficacy when a drug is administered in a liposomal formulation as compared to the free drug. However, potential toxicity issues have not been explored in detail. And yet, this may become the decisive factor for the application of liposomal formulations in the clinical setting. And last, most liposomal formulations need to be given iv or ia, and therefore need a hospital setting. Hospitalization entails expenses on the one hand, and may be a burden for the patient on the other hand, which can be considered a significant hurdle by drug marketers.

Clearly, if there were a place for novel iv liposomal products in the treatment of RA, it should be in phase III of the treatment strategy as outlined by the EULAR (Figure 1) besides the biologicals, but only if a clear advantage can be shown at the level of therapeutic index and/or at the level of treatment costs. There might also be a more restricted place for novel iv liposomal products in phase II of the EULAR RA treatment strategy if the liposomal product can be used in an intervention setting to induce a remission during a phase of active RA in a patient who is otherwise stable on relatively cheap generic DMARDs. In this situation an effective liposomal product may help keep a patient in this phase, preventing the switch to the more expensive third phase.

Liposomal formulations for local, intra-articular RA therapy fall a bit outside this EULAR treatment strategy discussion. Here the question is purely whether or not liposomal encapsulation of the agent results in an increased and prolonged effect of the incorporated drug without causing any safety issues. The market for such a product is likely limited to only those cases in which only one or a few joints are severely affected.

Concluding, while the advances in the field of liposomal drug delivery in RA as reviewed in this contribution are encouraging, one should be careful about claiming great expectations on the basis of these achievements for the future of the management of RA. Clearly, additional (pre)clinical research is mandatory to demonstrate clinical and commercial application in RA therapy.

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ABBREVIATIONS USED

AA, adjuvant arthritis; Ab, antibody; AIA, antigen-induced arthritis; BAL, bioadhesive liposomes; BMHS, betamethasone hemisuccinate; BNCT, boron neutron capture therapy; BPD-MA, BPD-verteporfin; BSP, betamethasone sodium phosphate; BUP, budesonide disodium phosphate; C8E4, *N*-octyltetraoxyethylene; C-940, Carbopol 940 (cross-linked PAA); cationic lipid RPR209120, (2-(3-[bis(3-aminopropyl)amino]propylamino)-*N*-ditetradecylcarbamoylmethyl-acetamide; CHEMS, cholesterol hemisuccinate; CHOL, cholesterol; CIA, collagen-(type II)-induced arthritis; CMC-Na, carboxymethyl cellulose; COL, collagen; COMP, cartilage oligomeric protein; COX, cyclooxygenase; cRGD, cyclic Arg-Gly-Asp motif containing peptide; DCP, diacetyl phosphate; DEX, dexamethasone; DFNa, diclofenac sodium; DMARD, disease modifying antirheumatic drug; DMGP, 1, 2-dimyristoyl-*sn*-glycero-3-phosphate; DMP, dexamethasone palmitate; DMPC, dimyristoyl phosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DOGS-NTA, 1,2-dioleoyl-*sn*-glycero-3-{[*N*-(5-amino-1-carboxypentyl)iminodiacetic acid]succinyl}(nickel salt); DOPE, dioleoyl phosphatidylethanolamine; DPPA, dipalmitoyl phosphatidic acid; DPPC, dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl phosphatidylethanolamine; DSPC, distearoyl phosphatidylcholine; DSPE, distearoyl phosphatidylethanolamine; DSPG, distearoyl phosphatidyl-glycerol; DXM, dexamethasone 21-acetate; DXP, dexamethasone disodium phosphate; ec, epicutaneous; EL, egg lecithin; EPC, egg phosphatidylcholine; EPR, enhanced permeability and retention; EULAR, European League Against Rheumatism; FudR-dP, dipalmitate-5′-fluorodeoxyuridine; GC, glucocorticoid; HA, hyaluronan; HSPC, hydrogentated soybean phosphatidylcholine; ia, intra-articular; IL, interleukin; ip, intraperitoneaal; iv, intraveneous; Lf, lactoferrin; LUV, large unilamellar vesicle; mAb, monoclonal antibody; MLV, large multilamellar vesicle; MMP, matrix metalloproteinase; MPS, mononuclear phagocyte system; MPHS, methylprednisolone hemisuccinate; MTX, methotrexate; MTX-DMPE, methotrexate-γ-dimyristoylphosphatidylethanolamine; NSAID, nonsteroidal anti-inflammatory drug; OA, osteoarthritis; OLV, oligolamellar vesicles; PA, phosphatidic acid; PAA, poly(acrylic acid); PC, phosphatidylcholine; PEG, poly(ethylene glycol); PEG-S, poly(ethylene glycol) MS400 stearate; PHEA, poly(hydroxyethyl L-asparagine); PLP, prednisolone disodium phosphate; PLP-PEG-L, PLP encapsulated in PEG-liposomes; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PS, phosphatidylserine; PVA, poly(vinyl alcohol); RA, rheumatoid arthritis; ROS, reactive oxygen species; SA, stearylamine; SCW, streptococcal cell wall; SDS, sodium dodecyl sulfate; siRNA, small interfering RNA; SLX(-L), sialyl-Lewis X (coated liposomes); SM, porcine brain sphingomyelin; SOD, superoxide dismutase; SOD-PEG-L, SOD encapsulated in PEG-liposomes; SPC, soybean phosphatidylcholine; SUR I, surfactant I (polyglyceryl-3-cetyl ether); SUV, small unilamellar vesicles; TAC, triamcinolone acetonide; TAC-P, triamcinolone acetonide 21-palmitate; Tf(s), transferosome(s); TNFα, tumor necrosis factor alpha; TRX-20, 3,5-dipentadecyloxybenzamidine hydrochloride; VECs, vascular endothelial cells

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