Expert Opinion

Drug delivery systems for the treatment of rheumatoid arthritis

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Rheumatoid arthritis (RA) is a severe immune-mediated disease characterized by chronically progressive inflammation and destruction of joints and associated structures. Significant advances in our understanding of its pathophysiology and early diagnosis have led to improved therapy and better outcome. Nevertheless, a number of details in the pathogenesis of RA are still unknown and thus the disease cannot be cured at present. Therefore, current therapy aims at accomplishing complete and long-lasting remission. However, this goal is only achieved in a small proportion of patients, and partial remission and frequent relapses are a common problem. A significant number of patients still do not respond at all to available treatments. In addition, all antirheumatic and immune-modulating drugs developed so far carry a considerable risk of adverse effects, some of which can be severe or even life threatening. This is due, at least in part, to a lack of specificity of most drugs for the target tissue, and to a high volume of distribution for systemic application, which, together with rapid clearance of most drugs, requires frequent application of high dosages. Targeted drug delivery and prolongation of bioavailability would alleviate this issue significantly. This article, therefore, reviews a selection of studies that report promising strategies for joint specific delivery of antiarthritic drugs.

Keywords: drug delivery, drug modification, liposomes, local delivery, nanoparticles, rheumatoid arthritis, targeted therapy

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1. Introduction

1.1 Rheumatoid arthritis

Rheumatoid arthritis (RA) is an immune-mediated chronic inflammatory disease that is characterized by chronic progressive inflammation and subsequent destruction of peripheral joints, the spine, bursae, ligaments and tendons. In addition, several extraarticular manifestations can occur [1]. The pathogenesis of RA has not been fully elucidated yet, but considerable progress has been made in understanding this disease over the last 50 years. Inflammatory activation of the synovium, the lining tissue of diarthrodial joints, tendon sheaths and bursae, represents the core process in RA pathology [2,3]. Infiltration by inflammatory cells, release of pro-inflammatory cytokines and chemokines, and abnormal activation of synovial fibroblasts result in joint swelling and pain, which are the clinical hallmarks of RA. These processes lead to progressive degradation of articular cartilage and subchondral bone mediated by the release of matrix-degrading enzymes and activation of osteoclasts, resulting in joint destruction and disability.

RA is a frequently occurring disease, a debilitating disease and a still insufficiently understood disease, thus constituting a particular therapeutic challenge. Nevertheless, its importance and impact are frequently underestimated [4].

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Although RA is the most common rheumatic disease with a worldwide prevalence of 1% of the population and a mean incidence of 0.03% [1,4,5], it is often regarded as a rare disease. Likewise, it is commonly regarded as a disease of older people, whereas it occurs primarily in individuals aged 40 – 70 years. In addition, young adults, adolescents and even children can also be affected. Furthermore, RA is commonly classified as a non-lethal disease. However, the natural course of disease leads to inflammatory destruction of the peripheral joints and the spine, resulting in severe disability and a higher risk of accidents. Extraarticular manifestations such as vasculitis, cardiac, pulmonary and renal involvement, as well as an increased incidence of comorbidities and complications including cardiovascular disease, lymphoproliferatve disorders and infections, are the causes of an increased mortality and a reduction in mean life expectancy by 5 – 10 years [4,6,7].

Taken together, these facts illustrate that RA represents a significant medical, social and economic burden [4,8]. The pivotal issue in relieving this burden is the development of effective and safe means of therapy.

1.2 Therapeutic challenges in rheumatoid arthritis

With an increasing understanding of the pathogenesis of RA, a broad variety of therapeutics, particularly disease-modifying antirheumatic drugs (DMARDs) have been developed. In particular, following elucidation of the importance of cytokines, there has been a burst of new drugs over the last 15 years, primarily biologic agents targeting these cytokines [9]. At present, a number of biologics are in clinical trials or in clinical development [10].

This impressive progress has supplied clinical rheumatologists with a decent therapeutic armamentarium that has the potency to induce disease remissions and prevent joint destruction. However, current therapy of RA is still challenged by several issues. These include lack of specificity, incomplete understanding of the mechanisms of action of many DMARDs, limited half-life, toxicity, high cost, and lack of efficacy in a significant proportion of patients.

The so-called conventional DMARDs, such as methotrexate, sulfasalazine, ciclosporin A, azathioprine, (hydroxy-)chloroquine, cyclophosphamide and leflunomide, are non-specific broad-range immunosuppressants, but the precise mechanism of these is understood only to a limited extent. Because application is usually systemic, not only target tissues, but also other organs and tissues are exposed to significant amounts of drug causing adverse effects. This also hampers the use of non-steroidal anti-inflammatory drugs (NSAIDs), particularly the non-selective cyclooxygenase 1 and 2 inhibitors, accounting for their gastrointestinal toxicity. The novel biologic agents are more specific as they are designed to target defined molecules such as TNF or biologic processes such as cellular costimulation. However, similar to broad-range immuno-suppression, systemic inhibition of these key immunological processes carries a high risk of infectious complications [11].

In addition, the pharmacokinetics of systemic application are frequently unfavorable. Orally administered agents depend on gastrointestinal resorption and are subject to first-pass metabolism in the liver. Parenteral application can result in a short plasma half-life due to clearance by first-order kinetics. Corticosteroids are a good example of this phenomenon. Corticosteroids are highly effective for rapid attenuation of inflammation and can even be regarded as a DMARD as they are capable of halting bone erosions [12,13], possibly by reducing synovial levels of receptor activator of nuclear factor kappa-B ligand (RANKL) [14]. When added to other DMARDs, corticosteroids can improve clinical and radiographic outcome, as indicated by the BeST study [15]. However, rapid clearance and an *a priori* high volume of distribution account for low concentrations of this class of drugs at the target sites and necessitate frequent application at high single doses. This results in high cumulative doses that are responsible for the adverse effects of glucocorticoids such as osteoporosis and diabetes.

Intraarticular injection is a simple means of achieving higher local levels of drugs such as corticosteroids in comparison with systemic application. Nevertheless, rapid clearance of unmodified drugs from the joint cavity can impair the effectiveness of this treatment strategy as well [16]. Furthermore, in active polyarticular disease, local injection of all affected joints is neither practical for the therapist nor tolerable for the patient.

High dosage and repeated application of antiarthritic drugs are also a cause for high treatment costs. The biologic agents are even more expensive due to high developmental costs and an elaborate manufacturing process. For those drugs that need to be administered intravenously such as infliximab, abatacept or rituximab, application costs have to be added.

Serious adverse events and high treatment costs can be tolerable to a certain extent provided that adequate therapeutic efficacy is achieved. Despite an overall good efficacy, particularly when using combination treatment, there is still a significant proportion of RA patients who do not respond to treatment satisfactorily.

Taking these issues together, an ideal treatment for RA would yield maximal efficacy at minimal dosage, have a prolonged half-life and molecular stability *in vivo*, accumulate preferentially at the sites of inflammation and joint destruction, target specifically the key mechanisms of arthritis without resulting in non-selective immunosuppression, have low systemic toxicity and be affordable.

Many, but not all, of the issues outlined above depend ultimately on the mode and route of delivery. Innovative delivery methods and delivery vehicles can provide improved stability, extended bioavailability, and optimized delivery to inflamed synovium. Thus, the effective dose will be increased, whereas the total dosage can be reduced. As a consequence of targeted delivery and lower total dosage, the risk for

adverse events will also be minimized. Therefore, novel strategies for targeted drug delivery to inflamed synovium in RA have the potential of coming close to an ideal treatment, and a selection of promising examples from current research and development is discussed below.

2. Targeted delivery strategies to inflamed synovium

In order to achieve its goal as a close-to-ideal treatment, there are three main requirements to be fulfilled by a delivery strategy for antiarthritic drugs. First, it should afford stabilization and extended bioavailability of the drug. This requirement can be achieved by molecular modification of the drug or the use of delivery vehicles. Second, it should shuttle the drug to the sites of inflammation, primarily the inflamed joints, with high specificity and efficacy. For this purpose the delivery vehicles need to be modified. Third, the drug should be released at the target site efficiently and preferably in a controlled manner. This depends on special characteristics of the delivery vehicle as well as specific molecular mechanisms. Examples of delivery vehicles and delivery tricks are presented in the following sections and summarized in **Table 1**.

2.1 Delivery vehicles

A variety of delivery vehicles have been developed and tested *in vitro* and *in vivo* for the delivery of small molecule drugs, peptide/protein drugs or therapeutic oligonucleotides and genes. Suitable vehicles include macromolecules that can be conjugated to the drug of choice, cells for *ex vivo* modification and adoptive transfer, or non-cellular artificial particles.

Regarding bioavailability of drugs and therapeutic molecules, delivery vehicles can protect them from interaction with opsonizing plasma proteins such as complement factors and immunoglobulins, from enzymatic degradation, and from renal or macrophage-mediated clearance.

An example of therapeutic molecules for which this kind of protection is particularly important is small interfering RNA (siRNA) [17]. Due to their low molecular weight (~ 13,000) and polyanionic charge, siRNA molecules are rapidly cleared by glomerular filtration in the kidneys. Furthermore, they are subject to enzymatic degradation in the circulation by serum nucleases. Stabilization can be achieved by conjugation to macromolecules such as cholesterol or polyethylene glycol (PEG) or encapsulation into artificial particles [17].

2.1.1 *Liposomes*

Liposomes are the most frequently used artificial particles, dating back to the 1970s [18], and are approved already for clinical use in humans. They are 80- to 100-nm vesicles composed of amphiphilic phospholipids and cholesterol forming a cell membrane-like bilayer that encapsulates a hydrophilic core [19].

This composition makes the liposomes versatile vehicles as they can encapsulate hydrophilic drugs in their aqueous core or accommodate lipophilic agents by integration into the bilayer. The fact that each liposome has the ability to carry several therapeutic molecules improves therapeutic efficacy in comparison with macromolecule conjugation or free drugs. In addition, other molecules can be bound to the liposomal surface for the purpose of improving pharmacokinetics and site-specific targeting. PEG, poly(amino acid)s (PAA), and other hydrophilic macromolecules are typically used to protect liposomes against plasma protein interaction and phagocytosis by the reticuloendothelial system [19,20]. Such sterically stabilized liposomes are also referred to as stealth liposomes.

The therapeutic usefulness of liposomal vehicles critically depends on several parameters, including the vesicle size, lipid composition and membrane charge. These parameters influence drug loading capacity, biodistribution and tolerability.

In a study by Metselaar and co-workers, large liposomes (450 – 500 nm diameter) showed higher accumulation in the liver and spleen and lower accumulation in inflamed paws in the adjuvant arthritis model in the rat in comparison with small (90 – 100 nm) liposomes [21]. Among the small liposomes, the PEGylated variant exhibited the lowest splenic uptake and highest targeting to joint tissue. Thus, this carrier variant, which encapsulated prednisolone phosphate as the target drug, achieved the best therapeutic effect [21].

Shehata *et al.* [22] were able to show that a combination of PEG and polyvinyl alcohol (PVA) at a molar ratio of 4:1 for surface modification of liposomes affords improved bioavailabilty. Compared with liposomes that were modified by PEGylation alone, their PEG/PVA constructs were less prone to opsonin binding and receptor-mediated endocytosis in the liver.

For liposomes formulated with PAA as stealth coating, a study by Romberg *et al.* [20] demonstrated that the grafting density of PAA and the cholesterol content-dependent membrane fluidity did not affect the liposomal circulation time significantly. In contrast, a high content of either negatively charged phospholipids, particularly phosphatidylserine, or positively charged phospholipids, such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), as well as particle size > 150 nm significantly enhanced liposomal clearance.

The net charge of liposomes not only has an influence on clearance by the reticuloendothelial system, but also on immunologic tolerability. Cationic liposomes used for the application of siRNA have been reported to induce type I and type II interferon responses [23], although this phenomenon was not observed in another study using a carrier DNA to complex siRNA with cationic lioposomes [24]. Anionic liposomes on the other hand can activate the complement system and thus cause a potentially life-threatening hypersensitivity reaction with cardiorespiratory distress, as has been observed in cancer treatment with PEGylated liposomal doxorubicin [25,26]. A complement-activating anionic charge

Table 1. Synopsis of delivery vehicles and delivery strategies.

Delivery vehicle	Delivery trick	Delivered drug	Ref.
Liposomes	EPR effect: PEGylation and small size (90 – 100 nm) facilitate less splenic accumulation and higher uptake in joints than non-PEGylated or larger liposomes	Prednisolone	[21]
Liposomes	EPR effect: combination of PEG and PVA at a molar ratio of 4:1 protects against opsonin binding and receptor-mediated endocytosis	None (methodological paper)	[22]
Liposomes	EPR effect: complexation of siRNA with carrier DNA	TNF siRNA	[24]
Liposomes	EPR effect: PEGylation	Prednisolone	[27]
Liposomes	Angiogenesis: targeting of liposomes to $\alpha_v\beta_3$-integrin on vascular endothelial cells by RGD peptide	Dexamethasone	[28]
Liposomes	EPR effect: expression of active enzymatic activity on liposomal surface facilitates therapeutic activity of liposomes before disruption as well as sustained release	Superoxide dismutase	[29]
Liposomes	EPR effect: remote loading technique affords higher loading efficiency and controlled release	Methylprednisolone	[30]
Nanoparticles	EPR effect: prolonged release	Betamethasone	[34]
Dendrimers	EPR effect and folic acid: PEGylation and folate coupling	Indomethacin	[35]
Macromolecules	EPR effect: albumin coupling	Methotrexate	[37-39]
Bioreductive delivery systems	Hypoxia-induced bioreduction leading to drug release	None (methodological paper)	[42]
HPMA-copolymer	pH-sensitive drug release	Dexamethasone	[44]
Specific antibody	Angiogenesis: immunotargeting of vascular endothelial cells using an anti-E-selectin antibody Fab fragment	None (molecular imaging study)	[48,49]
Specific antibody	Angiogenesis: targeting of vascular ECM components that are expressed due to alternative splicing during matrix remodeling in angiogenesis using specific antibodies	IL-10	[52]
Phages displaying random peptides	Arthritis-specific antigen: *in vivo* phage display as a strategy for the identification of synovium-specific antigens	None (methodological paper)	[57]
Complement receptor	Complement: targeting of long-lived complement factor C3 cleavage products (e.g., iC3b, C3dg, C3d) that are deposited at sites of inflammation using CR2	Soluble CR1	[60]
Specific antibody	Specific surface receptors: depletion of $CD64^+$ synovial macrophages using anti-CD64-coupled toxins	Ricin A or calicheamicin	[62-64]
Specific antibody	Specific surface receptors: depletion of FRβ expressing synovial macrophages using anti-FRβ coupled toxins	*Pseudomonas* exotoxin A	[65-67]

CR2: Complement receptor 2; ECM: Extracellular matrix; EPR: Enhanced permeability and retention; FRβ: Folate receptor β; HPMA: *N*-(2-hydroxypropyl) methacrylamide; IL-10: Interleukin-10; PEG: Polyethylene glycol; PVA: Polyvinyl alcohol; siRNA: Small interfering RNA.

can be due to either a high content of the liposomal membrane of anionic lipids or the presence of a net negative charge localized on the phosphate oxygen moiety of a methoxyPEG stealth coating [26]. By contrast, neutral liposomes as well as liposomes conjugated with methylated methoxyPEG do not cause complement activation [25,26]. Liposomes with a high cholesterol content > 30 mol% can also activate complement via the classical pathway after binding of anticholesterol antibodies, which are abundant in most human sera [26].

These findings illustrate that the use of liposomes for routine application in the treatment of RA in humans requires very careful engineering, and further research is needed to achieve the necessary fine-tuning of liposomal vehicles. In addition, it has been reported recently that PEGylated liposomes and even non-PEGylated liposomes can induce the generation of anti-PEG IgM antibodies after administration for the first time, and that these antibodies are responsible for accelerated liposomal clearance following repeated dosing. Consequently, ongoing liposome research has to address this issue as well.

Nevertheless, there are convincing preclinical data from animal studies using liposomes that demonstrate significant improvement of arthritis in comparison with conventional application of free drug [21,24,27-30].

The majority of studies used liposomes for the application of a corticosteroid [21,27,28,30]. As outlined above, long-term

use of corticosteroids for the treatment of RA is associated with severe adverse effects that are correlated with the cumulative dose. Although different corticosteroid molecules and different liposomal formulations were used, all studies showed that one or two injections of liposomal corticosteroids were sufficient for achieving almost complete clinical remission of rat adjuvant-induced arthritis (AIA) and murine collagen-induced arthritis (CIA), whereas treatment with equivalent doses of free drug had no effect. On daily administration, free drug was only as effective as a 10-fold lower dose of liposomal drug [27]. Even single treatment with the liposomal corticosteroid significantly reduced cartilage degradation in the CIA model [27]. Given this efficacy, the use of liposomal formulations of corticosteroids in humans could allow a significant reduction of the cumulative dose and thus markedly improve therapeutic use and safety of this potent class of drugs.

Because liposomes are commonly used for gene transfer into cells *in vitro* it is reasonable to try them as vehicles for intracellular delivery of siRNA *in vivo*. If delivered efficiently to their target cells, inhibition of gene expression by siRNA is regarded to be highly selective and more efficient than antisense strategies [17]. Khoury and co-workers reported almost complete and long-lasting clinical remission of CIA as well as significantly improved radiologic and histologic scores following repeated injections of liposomal siRNA targeting TNF [24]. Further analysis yielded a 50 – 70% inhibition of local articular and systemic levels of TNF as well as a significant reduction of local and systemic levels of IL-6 and monocyte chemoattractant protein-1. These effects were strongly enhanced by using carrier DNA for complex formation of the siRNA with the liposomes. Other investigators have used liposomal clodronate for the depletion of synovial macrophages with good success [31,32], or liposomal methotrexate (MTX), the gold standard in current RA therapy, for the treatment of AIA with increased efficacy and reduced hematopoietic toxicity [33].

All of these studies were able to demonstrate that, although the majority of liposomes are eventually taken up by the liver and spleen, there is noticeable accumulation of the liposomally formulated drugs in inflamed joints as opposed to non-inflamed joints [21,24,27,29,30]. This phenomenon is generally attributed to the enhanced permeability and retention (EPR) effect that occurs in inflamed tissues and tumor tissues as a result of increased capillary leakiness due to the effects of pro-inflammatory mediators and angiogenesis of structurally abnormal vessels. In addition, there are data to suggest that the liposomes are endocytosed by systemic macrophages subsequently entering the joints as well as by local macrophages in the joints [27].

2.1.2 *Other synthetic particles*

As alternatives to liposomes, dendrimers and solid polymeric nanopaticles have been developed for drug delivery. Dendrimers consist of repeatedly branching polymers forming a fractal-like structure of 10 – 100 nm in size [19]. Again, PEG is frequently attached to these molecules. The large surface of the branched molecules as well as attached PEG allow binding of a large payload of drug to dendrimers, while the small total size affords efficient internalization by target cells. Solid polymer particles are composed of a blend of hydrophobic and hydrophilic biodegradable polymers that can encapsulate therapeutic molecules [19]. Depending on their composition, nanoparticles facilitate slow, extended release of the encapsulated drug over a period of days or weeks.

Higaki *et al.* [34] used poly(D,L-lactic/glycolic acid) (PLGA) nanoparticles to deliver the hydrophilic corticosteroid betamethasone sodium phosphate (BSP) in rat AIA and murine antitype II collagen antibody induced arthritis (AbIA). In analogy to the abovementioned treatment approaches with liposomes, the nanoparticle-encapsulated BSP at a single dose of 100 µg was superior to a threefold dose of free BSP with regard to paw swelling and synovial infiltration by inflammatory cells. Of note, cartilage erosion could not be prevented in the AIA model, but the authors did not report whether the free drug and vehicle control groups exhibited significantly more erosion. Approximately 95% of the nanospheres located to the liver and spleen, whereas the remainder accumulated preferentially in inflamed versus non-inflamed joints. Thus, the delivery of BSP by these vehicles was only moderately targeted. Yet, systemic immunosuppression and elevated serum levels of free betamethasone were not detected, thus indicating that this treatment strategy may still have a favorable safety profile.

PEGylated dendrimers were used by Chandrasekar *et al.* [35] for the delivery of the NSAID indometacine in rat AIA. Indometacine is a potent antiphlogistic drug that has proved to be particularly effective for the alleviation of pain in RA, psoriatic arthritis and ankylosing spondylitis. However, its use is limited by the risk of gastrointestinal ulceration and renal toxicity. Dendrimer-associated indometacin was found to accumulate in inflamed joints in AIA at a significantly higher concentration than free indometacin, which the authors attributed to the EPR effect in arthritis. At the same time, accumulation of dendrimer–indometacin in the stomach was even significantly higher than that of free drug [35]. This disadvantage could be overcome when folate was conjugated to the dendrimer vehicles. Accumulation in inflamed paws was thus enhanced compared with unmodified dendrimers, whereas the concentration of indometacin in stomach tissue was reduced significantly. As discussed below, conjugation to folate is one of several promising options for improving tissue specific delivery.

2.2 Delivery targets and delivery 'tricks'

For improved delivery of therapeutic agents to inflamed joints in RA, several unique characteristics and pathophysiological phenomena of this disease can be used either as targets or as tools for drug delivery. These include the EPR effect of inflamed tissues, synovial accumulation of

inflammatory cells such as macrophages and T lymphocytes, enrichment of inflammatory mediators such as cytokines and chemokines, tissue hypoxia and acidosis, and angiogenesis. In addition, considerable research efforts are undertaken in order to identify synovial tissue-specific antigens that could facilitate the direction of delivery vehicles to the inflamed joints.

2.2.1 *Macromolecules and the enhanced permeability and retention effect*

As mentioned above, the EPR effect allows macromolecules and artificial particles such as liposomes, dendrimers and solid polymers to extravasate easily, although non-specifically, into areas of inflammation. Due to this phenomenon albumin and other serum proteins extravasate into inflamed joints in increased amounts. Compared with normal joints, the permeability of inflamed joints for albumin is increased up to sixfold [36]. In addition, albumin can thus serve as a nutritional source for metabolically active cells in the inflamed synovium such as macrophages and synovial fibroblasts. Accordingly, albumin can serve as a carrier for anti-inflammatory drugs such as MTX. Human serum albumin (HSA)-conjugated MTX has been shown to be significantly more effective than free drug in preventing the onset of arthritis in the CIA model [37]. For equivalent efficacy, a fivefold higher dose of free MTX was needed compared with HSA-MTX in that study. However, with regard to cartilage destruction, HSA-MTX and free MTX were equally effective [38]. Because many adverse effects of MTX are dose-dependent, a fivefold dose reduction could increase the tolerability of MTX substantially.

The use of HSA is, however, associated with several disadvantages. HSA is manufactured from plasma donations requiring rigorous testing for pathogens, particularly hepatitis viruses and HIV. The yield is low and available amounts are limited. Consequently, HSA is relatively expensive. In addition, its capacity as a carrier for conjugated drugs is limited and less efficient than, for instance, liposomes. Thus, the production of large amounts of HSA-MTX is difficult and expensive. A smarter strategy has been published recently by Fiehn and colleagues [39] using a modified form of MTX that can bind to endogenous albumin after intravenous transfer. MTX was converted into a prodrug by conjugation to a polypeptide that binds selectively to the cysteine residue at position 34 of endogenous albumin. MTX is then released in inflamed joints by enzymatic cleavage. This approach is more economic than HSA-MTX and proved to be equally effective in the CIA model allowing for a fivefold dose reduction versus free MTX [39]. Furthermore, enzymatic cleavage by cathepsin B and plasmin, both of which are highly expressed in arthritic joints, should increase the selectivity of this treatment.

2.2.2 *Hypoxia and acidosis*

Progressive thickening of the synovial membrane in inflamed joints by cellular infiltration and proliferation of local cells, the associated increase of cellular metabolism as well as tissue compression by an increasing synovial fluid effusion in the joint cavity lead to tissue hypoxia and acidosis [40,41]. Both of these pathophysiologic changes can be used for drug delivery.

Several bioreductive delivery systems that release the drug under hypoxic conditions have been described for tumor therapy [42]. Many of these, such as the nitroaromatic heterocyclics or the indolequinones, are actual cytotoxics when reduced under hypoxic conditions. Although this is desirable for tumor therapy, non-toxic carriers are needed in RA treatment. Of interest, bioreductive carriers have been designed that will self-inactivate by intramolecular cyclization after release of the conjugated drug of choice thus avoiding the formation of cytotoxic DNA adducts. Examples of such self-inactivating constructs include quinone lactonization systems or vitamin E analogs.

In quinone lactonization systems, the drug is conjugated to a benzoquinone trigger by a propionic acid linker. Following reduction of the benzoquinone, a lactone is formed by intramolecular cyclization und the drug is thus released. Lactonization is the preferred reaction to acid-catalyzed reduction because the parent molecule carries three methyl groups that impart steric hindrance, the so-called trimethyl lock [42,43]. Vitamin E analogs use the fact that oxidized vitamin E undergoes cyclization on reduction, resulting in ejection of its hydroxy group. Thus, a therapeutic molecule conjugated to the hydroxy group of oxidized vitamin E can be released [42]. These strategies have been tested *in vitro* and in tumor models; their adaptation to use in arthritis models is still pending. Yet, the concept is very interesting and worthwhile pursuing.

A construct that has been tested in the AIA model is a pH-sensitive vehicle [44] based on an *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer that was found to accumulate in arthritic joints for 1 – 2 days depending on and correlating with disease activity [45]. The authors conjugated dexamethasone to the HPMA copolymer by a pH-sensitive hydrazone bond. *In vitro* analysis confirmed that the drug was best released at acidic pH (5.0) and body temperature (37°C). Interestingly, the release rate was 1% per day *in vitro*, whereas *in vivo*, the release must be accelerated because the onset of the effect of HPMA-conjugated dexamethasone was quick. Of note, HPMA-delivered dexamethasone at 2 mg/kg was more effective than an equal dose of free drug with regard to clinical arthritis activity as well as prevention of cartilage and bone erosion [44]. The authors concluded that this effect is due, to a large extent, to extracellular acidosis-mediated release of dexamethasone in the arthritic joints. Further evidence will be required to substantiate this hypothesis.

2.2.3 *Angiogenesis*

Tissue hypoxia leads to formation of new blood vessels, a process termed angiogenesis [46,47]. The (neo-)vascular

endothelial cells are thus prime targets for delivery strategies of antiarthritic drugs both as a stepping stone for access to the synovium as well as drug targets themselves for the inhibition of angiogenesis. In order to facilitate the physiological extravasation of inflammatory cells, vascular endothelial cells express a variety of different adhesion molecules. Two examples are $\alpha_v\beta_3$-integrin and E-selectin, both of which are markedly upregulated in inflammation. E-selectin has served as a target for synovitis-specific scintigraphic imaging, using ^{111}Indium or ^{99m}Technetium as tracers conjugated to an anti-E-selectin monoclonal antibody antigen-binding fragment (Fab) in pigs and in humans [48,49]. These studies illustrated a high and specific synovial tissue uptake of the tracer compared with an isotype control and thus suggest E-selectin as a valuable target for drug delivery. However, treatment studies in arthritis using this delivery strategy are missing.

By contrast, the $\alpha_v\beta_3$-integrin has been used successfully for a targeted delivery strategy. Cyclic peptides containing an Arg-Gly-Asp (RGD) sequence can bind this integrin specifically [50]. Koning and co-workers [28] used a high-affinity RGD peptide to improve the targeting of dexamethasone phosphate carrying PEGylated liposomes. Improved uptake of RGD-conjugated PEG-liposomes versus unmodified PEG-liposomes into inflamed tissues was validated by several methods. However, there was an accelerated clearance of RGD-modified liposomes from the circulation by hepatic uptake. Nevertheless, delivery of dexamethasone by these vehicles effectively reduced arthritis in the rat AIA model over a prolonged period of time. Even single administration of 1 mg/kg was sufficient compared with unmodified liposomal dexamethasone [28]. Free dexamethasone was not included as a control. The exact mechanism of the therapeutic effect in this study is unknown, but modulation of the vascular endothelial cell function has been hypothesized, as steroids have a known antiangiogenic effect and inhibit the expression of leukocyte adhesion molecules on endothelial cells. After binding of the RGD peptide, the liposomes are taken up by endothelial cells and the liposomally encapsulated dexamethasone phosphate is processed in the endocytic compartment into active dexamethasone, which, as an amphiphilic molecule, can pass through the endosomal membrane into the cytosol and exert its effects. This study demonstrates that the enhanced efficacy of liposomal corticosteroids can be improved even further by this targeting strategy.

2.2.4 *Arthritis-specific antigens*

During the process of angiogenesis, the extracellular matrix is remodeled by proteolysis of existing structures and neosynthesis of its components, paving the path for endothelial cells to proliferate, migrate and align in tube-like structures thus forming the new vessels. During this remodeling process, certain extracellular matrix components that are usually not found in mature vascular structures are expressed due to alternative splicing. They thus represent therapeutic targets with a relative specificity for tissues with active angiogenesis [51]. Interesting examples include the C domain of tenascin-C and the extra domain B of fibronectin.

Specific antibodies have been developed against these domains, termed G11 and L19, respectively [52]. Of these, the L19 antibody has already been used with great success for *in vivo* imaging of angiogenesis and for delivery of conjugated therapeutic molecules in tumor models [53-55]. A recent study investigated the homing capability of both antibodies in the CIA model [52]. Immunohistochemistry confirmed the presence of the antigens in inflamed synovial tissue, and fluorescence imaging *in vivo* as well as autoradiography post mortem revealed selective accumulation in inflamed versus non-inflamed paws. The enrichment of L19-conjugated tracers in inflamed paws was up to 8.5-fold higher than in non-inflamed paws. For the G11 conjugates the ratio was up to fivefold. Unfortunately, biodistribution of the tracers to the joints in comparison with other organs was not assessed in this study. The authors only presented data from an earlier study demonstrating low distribution of an L19-bound tracer to internal organs, whereas a substantial accumulation occurred in a subcutaneously implanted teratocarcinoma that was between 7- and 128-fold higher than in other tissues and organs [56]. Using an L19-IL-10 fusion construct, Trachsel *et al.* were able to show a significant inhibition of clinical disease progression in established CIA that was superior to non-targeted IL-10 [52]. This study is of particular interest because the extra domain B of fibronectin is identical in mice and humans and is strongly expressed in human RA, but not in healthy adult tissues. Thus, the L19 antibody has considerable potential for use in humans.

Considering these findings, other arthritis-specific antigens that could be targeted by monoclonal antibodies would strongly enhance the therapeutic options in RA. Thus far, the search for specific synovial antigens in arthritis has been largely elusive. However, a promising approach for the identification of such antigens by *in vivo* phage display has been published recently [57]. Phage display is a powerful technique that uses virion particles to express randomly generated libraries of peptides or antibodies (up to 10^9 permutations) for screening against known or unknown ligands. Bound phage can be isolated and amplified. Repetition of this process for several cycles results in enrichment of phage-expressed binding sequences. When applied *in vivo* by intravenous injection, this technique can yield vascular endothelial binding sequences that are specific for different organs of interest [58]. Application to humans is not possible for ethical reasons. However, Lee *et al.* took advantage of the SCID mouse model of arthritis that facilitates analysis of viable human joint tissue [59]. The authors were able to isolate homing peptides in human synovial implants in the SCID mice by multiple cycles of phage enrichment [57]. Although technically challenging and elaborate, this approach holds great promise for identification of arthritis-specific

ligands that could be tested for targeted delivery in the near future.

2.2.5 *The complement system*

The synovial inflammation in RA also causes other pathophysiologic phenomena that can be used for treatment delivery strategies, including local complement activation and enhanced infiltration by inflammatory cells.

Complement activation supports joint inflammation and, among other phenomena, leads to deposition of long-lived complement factor C3 cleavage products such as iC3b, C3dg and C3d. As complement receptor 2 (CR2) can bind to these cleavage products, Song *et al.* have constructed a fusion molecule consisting of CR2 as the targeting device and Crry, the murine analog of human soluble CR1, as the therapeutic molecule [60].

Free recombinant sCR1 has already been proved to be effective in rat CIA [61]. Although Song *et al.* did not compare their construct directly with free sCR1 in their study, they achieved substantial inhibition of clinical and histological disease progression and significant reduction of pannus formation, cellular infiltration and destruction of cartilage and bone. Of note, cytokine levels of TNF and IL-1β were significantly decreased, whereas IL-1 receptor antagonist and IL-10 were upregulated. This is particularly remarkable because retention of the construct in inflamed joints, although significantly higher than in non-inflamed joints, did not last for much longer than 48 h after single injection, and accumulation in liver, spleen and kidneys was equally high.

2.2.6 *Specific surface receptors*

Finally, inflammatory cells such as macrophages represent attractive targets as they play a pivotal role in arthritis pathology and can be identified by several surface molecules.

The high-affinity receptor for immunoglobulin G (FcγRI, CD64) is highly expressed on activated macrophages in synovial tissue and fluid, whereas CD64 expression on quiescent macrophages is low [62-64]. Thus, cytotoxic drugs such as ricin A or calicheamicin have been used to deplete activated $CD64^{high}$ macrophages *in vitro* and *in vivo* by conjugation to an anti-CD64 antibody [62-64]. Macrophage depletion *in vivo* in a model of human CD64 transgenic rats significantly diminished clinical arthritis severity as well as bone erosion.

Depletion of activated macrophages can also be mediated by uptake of folic acid-bound toxins via the folate receptor β (FRβ). Expression levels of FRβ are low on inactive macrophages, but high on activated macrophages in RA synovium [65,66]. Expression is also detected on tissue cells in brain, internal organs, lymphoid organs and skeletal muscle [65], but little or no uptake of folate is observed [66]. Application of truncated pseudomonas exotoxin conjugated to an anti-FRβ antibody *in vitro* and in the SCID mouse model *in vivo* caused a marked reduction of human macrophages [65,67]. Of note, in the synovial tissue implants in the SCID mouse model, synovial fibroblasts and endothelial cells were also diminished [67]. These results together with the fact that FRβ expression can be found in many tissues suggest that this approach, although effective, can be expected to be complicated by organ damage and profound immunosuppression.

Linkage of therapeutic agents to folic acid instead of anti-FRβ antibodies appears to be preferable because folate uptake by FRβ is more restricted. To this end, Paulos and co-workers used a folate-coupled hapten to eliminate activated macrophages and thus reduce arthritic inflammation and cartilage and bone degradation in the CIA model [68]. The disadvantage of this highly effective treatment is the necessity to immunize the animals with the hapten before folate-mediated therapy, which raises concerns with regard to human application.

Besides cell depletion, the ability of folic acid to target conjugated molecules to activated macrophages can also be used for other purposes. In the study by Chandrasekar *et al.* mentioned above, folate was conjugated to PEGylated dendrimers to improve the delivery of indometacin [35]. Similarly, folate can be bound to other molecules or liposomes to mediate drug transfer into activated macrophages.

3. Expert opinion

Taken together, the examples outlined above reflect a broad spectrum of tools, strategies and ideas for improved delivery of antiarthritic treatments in models of RA. Importantly, these studies illustrate two facts. First, there are a large variety of already quite advanced tools that have been developed most commonly in cancer research and can be adapted for use in RA. Second, there are a large variety of pathophysiologic features that are perhaps not unique, but very characteristic of RA and, therefore, can serve as targets or tools for improved therapy.

Delivery vehicles such as albumin, liposomes, dendrimers and polymer particles are highly promising for optimizing bioavailability of drugs. Hypoxia, acidosis, angiogenesis, local complement activation and infiltrating inflammatory cells have all been targeted successfully in animal models of RA. Many of the tools and strategies presented in this review still need some fine-tuning in terms of efficacy and safety before they can be applied in humans. Nevertheless, clinical trials appear to be not too remote for several treatments. Particularly, targeting of MTX and other DMARDs to endogenous albumin could be applied soon as could L19 antibody-coupled drugs. Furthermore, most liposomal formulations can be expected to advance rapidly, as liposomal anticancer drugs and contrast agents have already been approved for human use.

An important issue with regard to the large variety of different delivery vehicles and strategies is the lack of comparative data. For instance, a large number of different

liposomal formulations have been developed, all of which have been tested successfully against free drug and placebo. However, direct comparison of the different formulations in one experiment would be required in order to identify the most effective one for further development into human application.

Most delivery strategies quoted in this article are rather elaborate and routine application will therefore be relatively expensive. When reviewing drug delivery strategies for cathepsin inhibitors in joint diseases in a previous issue of this journal, Wang and Brömme [69] hypothesized that many novel, more elaborate and thus more costly treatment strategies may not be considered for further development because low molecular weight, orally available drugs are cheaper to produce, easier to administer and are associated with a higher patient compliance. The same may be true for the delivery strategies quoted here. However, as Wang and Brömme pointed out, the lack of efficacy and the safety profile of conventional DMARDs strongly underscore the need for alternative approaches.

Another concern in addition to cost and simplicity certainly is the safety of the new approaches. The associated risks such as complement-mediated hypersensitivity may not be regarded as justified in relation to the expected benefit. Other than malignant diseases, in which the research on targeted drug delivery has been advanced more vigorously, RA is still frequently regarded as a non-lethal disease. As outlined above, this notion is not correct. Rather, the arthritis-related disability, disease-related mortality and treatment-associated complication rate should result in stronger motivation for further research and translational studies on drug delivery in RA.

Given the recent insight into the mechanisms of complement-associated hypersensitivity reactions and the development of strategies to circumvent this problem, tolerability and safety will advance rapidly. Also, more selective tissue targeting of rheumatoid synovium is expected to develop from research into tissue- and vasculature-specific antigens such as *in vivo* phage display in the SCID mouse model of RA.

Furthermore, even with the non-optimized systems available today, significant improvements of RA therapy are possible. The remarkable reduction of the required cumulative doses of corticosteroids or MTX that can be achieved by using delivery vehicles such as liposomes or albumin already represents a quantum leap for safety and tolerability of these 'conventional' drugs. In conclusion, we expect significant advances in RA treatment efficacy and safety based on targeted delivery strategies within the next ten years for the benefit of our patients.

Declaration of interest

The authors declare no conflict of interest and have received no payment in preparation of this manuscript.

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