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Review

Drug targeting systems for inflammatory disease: One for all, all for one

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ABSTRACT

In various systemic disorders, structural changes in the microenvironment of diseased tissues enable both passive and active targeting of therapeutic agents to these tissues. This has led to a number of targeting approaches that enhance the accumulation of drugs in the target tissues, making drug targeting an attractive strategy for the treatment of various diseases. Remarkably, the strategic principles that form the basis of drug targeting are often employed for tumor targeting, while chronic inflammatory diseases appear to draw much less attention. To provide the reader with a general overview of the current status of drug targeting to inflammatory diseases, the passive and active targeting strategies that have been used for the treatment of rheumatoid arthritis (RA) and multiple sclerosis (MS) are discussed. The last part of this review addresses the dualism of platform technology-oriented ("one for all") and disease-oriented drug targeting research ("all for one"), both of which are key elements of effective drug targeting research.

Contents

1. Introduction

In the last decades, targeted drug delivery has become an established field in pharmaceutical research. By using a targeting system that assists in directing a drug to the site in the body where it needs to exert its effect, target tissue specificity of the therapeutic agent can be increased while the off target effects can be limited [1,2]. Although a drug targeting strategy can potentially improve the clinical efficacy of therapeutic interventions in many, if not all, diseases, most drug targeting research has been focused on cancer (Fig. 1) [3–5]. The high morbidity and mortality among cancer patients evidently justifies this focus working on tumor-targeted drug delivery systems. At the same time, the large socio-economical

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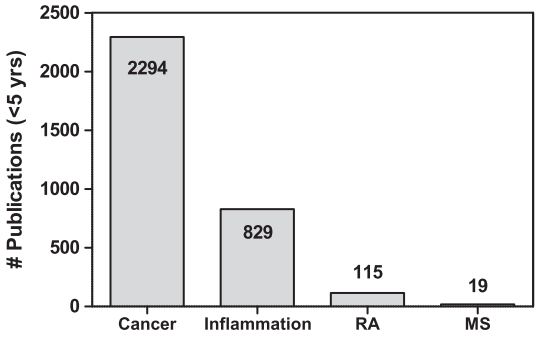

Fig. 1. Number of research publications over the last 5 years related to drug targeting to diseases. Results represent the number of hits of MEDLINE searches (query " drug delivery" or "drug targeting" or "nanomedicine"), specified to malignant diseases (using "cancer"), inflammatory diseases (using "inflamm*"), rheumatoid arthritis (RA, using "rheum*" or "arthritis"), and multiple sclerosis (MS, using "multiple sclerosis" or "encephalomyelitis").

impact of chronic inflammatory disorders, such as rheumatoid arthritis and multiple sclerosis, on both patient and society appears not to be fully appreciated in drug targeting research [6–8].

It is remarkable that there is only limited attention for these diseases, since in principle many strategies employed for targeted drug delivery to tumors would seem applicable for drug targeting to sites of inflammation. In fact, cancer is strongly linked to inflammation and is often designated as a chronic inflammatory disease itself, illustrating the overlap of cancer and inflammation in the context of drug delivery [9–11]. This contribution aims to provide the reader with an update of the current status of the field with respect to drug targeting in inflammatory disorders. In addition, we will give our perspective on how drug targeting can be approached to improve its clinical impact.

2. Drug targeting to inflammatory disease

2.1. Passive drug targeting

A quarter of a century ago, Maeda and coworkers demonstrated for the first time the tumoritropic accumulation of proteins and macromolecules [12]. By coupling poly(styrene-co-maleic acid) to a protein (neocarzinostatin) that has anti-tumor activity, a conjugate (SMANCS) with increased molecular weight was formed which showed an improved *in vivo* half-life compared to the unmodified protein [13]. To relate the efficacy of SMANCS to its target tissue concentration, the plasma clearance and tumor accumulation of neocarzinostatin, SMANCS and several other plasma proteins including albumin, were determined. A clear positive correlation between plasma half-life, molecular size and tumor-specific accumulation was observed, which was attributed to a 'highly enhanced leakiness' of the tumor vasculature for macromolecules [12]. Moreover, upon intratumoral injection of Evans blue-albumin complexes, there was a remarkable reduction of clearance of the complexes in the tumor compared to healthy tissues, indicating a tumor-specific deficit in lymphatic drainage. This phenomenon of enhanced vascular leakiness and impaired lymphatic drainage, now known as the 'enhanced permeability and retention (EPR) effect', has since been used extensively for passive tumor-specific drug delivery, also described as passive targeting, using macromolecular and particulate drug targeting systems [2,14–18].

However, the EPR effect has not been observed exclusively in tumors. In fact, in 1971, 15 years before the landmark study of Matsumara and Maeda, Kushner and Somerville described a similar relationship between the molecular size of proteins and their localization in arthritic joints of patients with rheumatoid arthritis (RA) and other arthritic diseases [19]. Although the precise mechanism remained unclear, one of the suggested mechanisms was an inflammation-induced 6- to 40-fold increase of blood–joint barrier permeability for high molecular weight molecules [20]. Consequently, a complication frequently observed in patients with RA is hypoalbuminemia, which may be attributed to an increased albumin extravasation and metabolism within the inflamed joint [21,22]. Similarly, an increase in blood–brain barrier (BBB) permeability for serum proteins, such as fibrinogen, has directly been correlated to areas of (active) demyelinization (i.e. plaques) in multiple sclerosis (MS) [23–25]. While the lymphatic drainage in inflamed tissues, when compared to tumors, appears to be still functioning [26], the significantly increased vascular permeability in the target tissues allows for the successful application of passively targeted drug delivery strategies in models of inflammatory diseases such as RA and MS [27–31].

It is important to emphasize that the size and the pharmacokinetic profile of the drug carrier are key characteristics of passively targeted drug delivery systems [32,33]. A lower size limit of ~50 kDa and an upper size limit in the range of ~200 nm enhance targeting of the carrier-associated drug by means of the EPR effect while preventing glomerular filtration [34,35]. The long circulation time of these carriers increases the statistical probability for sufficient target accumulation of the drug to take place. Indeed, significantly higher drug concentrations may be obtained in the target tissue by employing such passively targeted drug delivery systems, but the term 'targeted' may appear somewhat deceptive in this context [36,37]. Macromolecules and nanoparticulate carrier systems that are too large to be cleared renally from the body are taken up by phagocytic cells of the reticuloendothelial system (RES), mainly in liver and spleen [38]. As a result, by far the largest part of the injected dose is 'targeted' to these organs, while on average only a much smaller fraction (less than 10%) of the injected dose will end up in the tissue where the drug needs to exert its effect. Nevertheless, the therapeutic consequences of passive targeting (of macrophages) are likely more complex than the mere target tissue accumulation: there is, for example, evidence that the anti-tumor effect of liposomal glucocorticoids may be related to a decrease in white blood cells, rather than the accumulation in the target tissue [39].

2.2. Active drug targeting

While local drug concentrations in the diseased tissue can be increased by employing a passive targeting strategy, directing the drug delivery system to a specific cell type by means of a targeting ligand (i.e. active targeting) may help to further improve the efficacy of the targeted drug. Generally, such strategies do not increase the overall concentration in the target tissue, but rather change the distribution within the tissue. A notable exception in this case is targeting within the blood stream for which extravasation is not required and therefore not the rate-limiting step. In chronic inflammatory diseases such as RA and MS, a shortage of oxygen and nutrients induces the formation of new blood vessels, a process known as angiogenesis, which contributes to the pathogenesis and development of these diseases [40–45]. By interfering with the angiogenic process in preclinical models of RA and MS, it has been shown that the disease intensity can be alleviated [46–49]. Both vascular endothelial cells and monocyte-derived cells, including macrophages, are closely involved in the angiogenic process in chronic inflammatory diseases, which makes them attractive targets for an active drug targeting approach [50–54]. As a result of the pro-inflammatory microenvironment, membrane receptors that are involved in angiogenesis signaling are upregulated, marking the cells expressing them 'inflammation-specific', and designating them as possible targets for drug delivery [55].

Although several receptors are recognized as being suitable targets, primarily the folate receptor (FR) and the $\alpha_v\beta_3$ integrin have been used for active drug targeting purposes [56–58]. In 1991, Laemon and Low were the first to show that macromolecules, such as proteins, when conjugated to folate or folic acid were internalized *in vitro* by a number of different types of cells [59]. Later studies reported that the receptor mediating the uptake, i.e. the folate receptor, is overexpressed by several epithelial tumor cells and activated macrophages [60,61]. Since the tissue specific expression of FR makes it an attractive target, FR-directed drug targeting has developed into a mature strategy for active drug targeting [62–65]. FR-expressing cancer cells and activated macrophages express distinct FR isoforms, FR-α and FR-β, respectively [66,67], and much research has been focused on tumor-targeting via FR-α [68,69]. However, the potential of folate-functionalized drug delivery systems in the treatment of chronic inflammatory diseases by targeting FR-β expressed by activated macrophages should not be underestimated. In fact, there is evidence that the anti-tumor efficacy of FR-targeted drug delivery systems for cancer therapy is, at least partly, macrophage-mediated [70].

The $\alpha_v\beta_3$ integrin, a heterodimeric surface receptor expressed by several cells including endothelial cells and macrophages, enhances cell adhesion and migration of infiltrating cells during tissue inflammation [55]. Having a key role in angiogenesis, the $\alpha_v\beta_3$ integrin is only expressed on the luminal surface of endothelial cells that are associated with the neovascularization process, making these cells a specific target for anti-angiogenic therapy [71]. $A_v\beta_3$-targeted therapies that directly interfere with the binding of ligands to the receptor have shown efficacious angiogenesis inhibition and suppressing effects on disease development in models of both neoplastic and inflammatory diseases [72–74]. The strategies exploiting this integrin to target drugs to angiogenic tissues in tumors, as well as inflammatory diseases, often by using the cyclic Arg–Gly–Asp (RGD) peptide as a ligand, have been quite successful [58,75,76]. The strong similarities between active drug targeting approaches in cancer and those in chronic inflammatory diseases, as illustrated by the examples given above, emphasizes the importance of not focusing merely on a single disease but keeping a broad horizon regarding applications of a drug targeting system. In the following sections, several drug targeting strategies for the treatment of RA and MS are discussed in more detail.

2.3. Drug targeting in rheumatoid arthritis

RA is a systemic disorder characterized by a chronic inflammation in the synovium of one or several joints, initiated by an immunological response against a currently unknown endogenous or cross-reactive exogenous antigen [77]. Mediated by the release of proinflammatory cytokines and matrix metalloproteinases (MMPs) by various infiltrating immune cells, including macrophages, B cells, T cells, fibrocytes and synoviocytes (synovial fibroblasts), joint inflammation progresses into joint destruction [50,51,77,78]. In RA, activated synoviocytes exhibit invasive growth into the joint cartilage, and stimulate the differentiation and proliferation of osteoclasts responsible for joint destruction [78–80]. The activated synoviocytes are also considered to be responsible for the progression of the disease from one arthritic joint to other, unaffected joints, a role which bears resemblance to that of metastatic tumor cells in cancer [81]. Similarly, the activation of the vascular endothelium and its proliferation is comparable to the angiogenesis that occurs during tumor growth [40,42]. The enzymatic and osteoclastic destruction of the arthritic joint leads to joint deformation and loss of function, and to pain and morbidity for patients suffering from RA [77]. Moreover, although not regarded as a lethal disease, RA reduces the mean life expectancy of patients with 5–10 years, depending on disease severity [82,83].

There are several therapeutic strategies available in the clinical management of RA, aiming at the reduction of joint inflammation and the prevention of joint destruction [77,84]. To improve the efficacy of conventional therapies, a number of systemic drug targeting strategies taking advantage of the functional and cellular changes in the synovial inflammatory environment have shown to be been promising. The role of phagocytic cells in the clearance of systemically injected macromolecular and nanoparticulate drug delivery systems, as well as their importance in the development and progression of chronic inflammatory diseases, likely make macrophages and synoviocytes residing in the joint tissue important mediators in the therapeutic effect of targeted nanomedicines. In fact, several studies have investigated the effect of macrophage depletion on synovial inflammation using liposomes loaded with bisphosphonates, such as clodronate disodium, which induce cellular apoptosis when endocytosed [85–88]. Indeed, upon macrophage depletion joint inflammation was effectively suppressed. These studies confirmed that (activated) synovial macrophages and synoviocytes fulfill a key pro-inflammatory role in rheumatoid arthritis, and that these cells can be efficiently targeted by passively targeted drug delivery systems [85]. Over the years, a number of drug targeting systems containing several types of antirheumatic drugs have been prepared and evaluated in animal models for RA, as will be discussed further below.

2.3.1. Glucocorticoids

The therapeutic efficacy of glucocorticoids, which are frequently used in RA for suppressing exacerbations of joint inflammation, has been greatly enhanced upon encapsulation into long-circulating PEGylated liposomes [31,89–93]. For example, a single i.v. injection of 10 mg/mL prednisolone phosphate (PLP) encapsulated in liposomes almost completely resolved joint inflammation in rats with adjuvant-induced (AIA) arthritis, while 7 daily i.v. injections of free drug at the same dose only resulted in a mild reduction of the inflammation (Fig. 2B and C) [31]. Similar effects have been observed for the more potent corticosteroid dexamethasone phosphate (DXP) at lower doses in the same model [89], as well as in another, murine collagen-induced (CIA), model of RA [90]. By active targeting of DXP liposomes to the angiogenic endothelial cells in the inflamed synovium using RGD-functionalized PEG-lipids, their therapeutic efficacy could be further enhanced, even at early inflammatory stages , illustrating the versatility of this liposomal system [58]. In a study comparing the therapeutic index of liposomes encapsulating PLP, DXP and budesonide phosphate (BUP), in arthritic (AIA) rats, it was observed that BUP-liposomes possess the highest therapeutic efficacy, while showing the least systemic side-effects [91]. The beneficial effect of the glucocorticoid-loaded liposomes on joint inflammation is explained by the passive accumulation of the liposomes in the synovium of arthritic joints, which is not observed in healthy joints (Fig. 2A) [31,94].

This arthrotropic accumulation is not only seen for liposomes, but also for other nanomedicines. Both high-molecular-weight (>55 kDa) polymeric drug-conjugates, such as poly(*N*-(hydroxypropyl) methacrylamide) (pHPMA), and plasma albumin exhibited a comparable passive accumulation in arthritic joints, indicating that many types of macromolecules could serve as effective drug delivery systems for glucocorticoids and other agents for RA therapy (Fig. 2D) [95]. Indeed, joint inflammation, as well as arthritic bone resorption and joint destruction, could be strongly reduced in a number of arthritic rat models by systemic application of a pHPMA-conjugate carrying dexamethasone via a pH-responsive hydrazone linker (Fig. 2C and D) [30,96,97].

Using a similar strategy, several poly(ethylene glycol) (PEG)-conjugated hydrazone-linked prodrugs of dexamethasone were synthesized using different moieties of dexamethasone [98,99]. An interesting glucocorticoid-polymer construct consisting of α-methyl prednisolone (MP) coupled via ester-linkage to a linear cyclodextrin polymer, self-assembled into nanoparticles of around 30 nm, showed a significantly enhanced reduction of joint inflammation compared to free MP [100].

Another promising systemic approach for passive glucocorticoid delivery to arthritic joints in RA is the use of solid polymeric

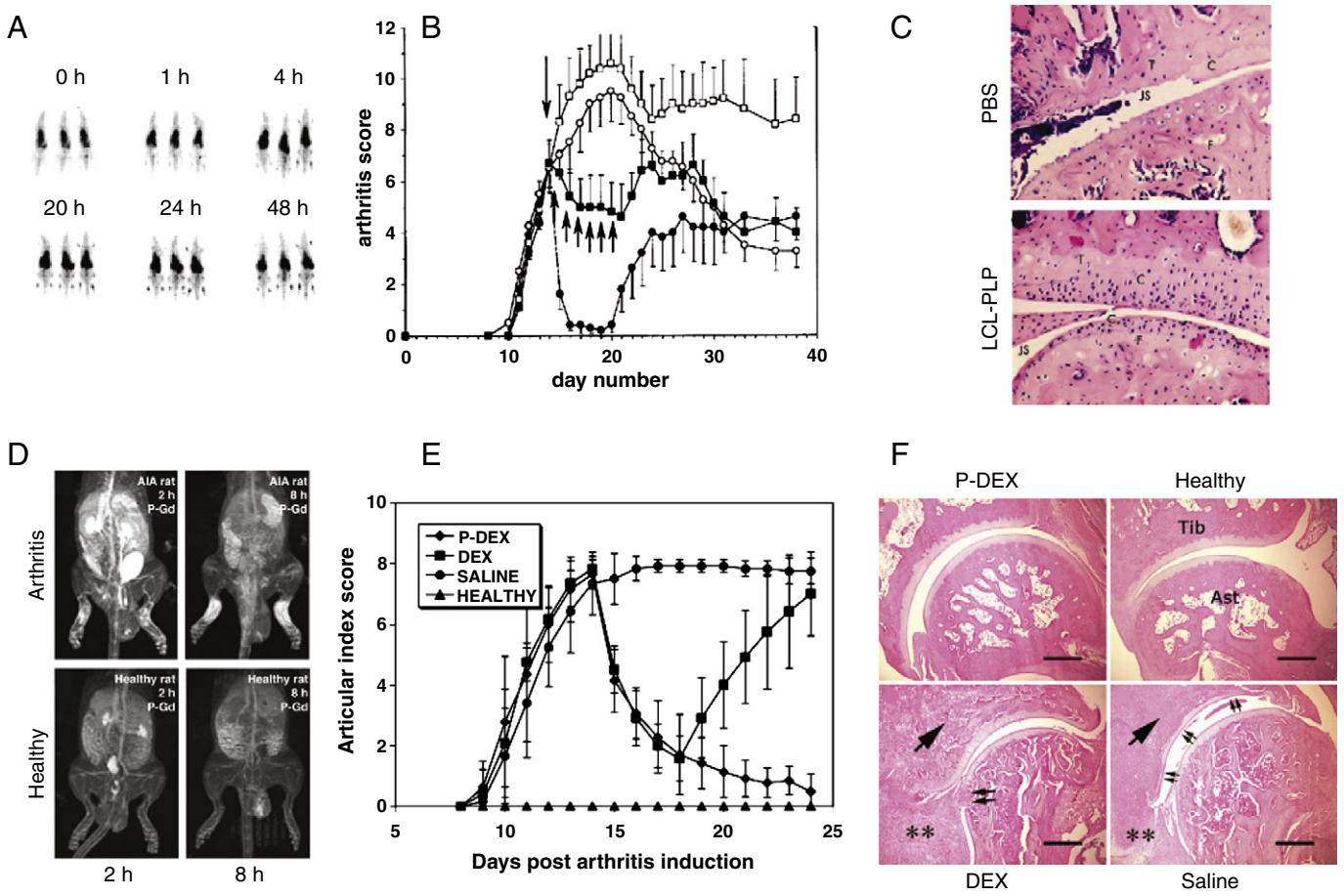

Fig. 2. Targeted glucocorticoids in RA. Glucocorticoids targeted to arthritic joints in rats using PEGylated liposomes (A–C) or pHPMA-conjugates (D–F). A. Whole body scintigraphic images showing the accumulation of ^{111}In-labeled PEGylated liposomes in the inflamed joints of rats with adjuvant-induced arthritis (AIA) up to 48 h post-injection [31]. B. Therapeutic activity of a single dose of PEGylated liposomes loaded with PLP (10 mg/kg, circles) on day 14, compared to 7 daily injections of free PLP (10 mg/kg, closed squares) on the clinical arthritis score. Rats treated with either saline (open squares) or PBS containing PEG-liposomes (open circles) presented with an increase in disease intensity during the days after injection. Upon daily treatment with free PLP, a stabilization of disease intensity was obtained, while a single injection of liposomal targeted PLP resulted in a nearly complete abolishment of paw inflammation, clearly illustrating the strong effect of targeting [31]. C. Histological staining of arthritic knees of rats with collagen-induced arthritis, 1 week after treatment with PBS or PEGylated liposomes loaded with PLP (LCL-PLP, 10 mg/kg). In the joints of rats treated with PBS, severe damage of the cartilage was observed (upper panel), while the joint cartilage of rats treated with PLP-liposomes appeared to be hardly affected (lower panel). T, tibia; F, femur, JS, joint space; C, cartilage layer. [94]. D. MR imaging of pHPMA-DOTA-Gadolinium conjugates in the inflamed joints of rats with AIA (upper panels) and the joints of healthy rats (lower panels). A clear accumulation of the pHPMA conjugates was observed 2 h (upper left) and 8 h (upper right) after i.v. injection, whereas no pHPMA conjugate accumulation could be observed in the joints of healthy rats after 2 h (lower left) or 8 h (lower right) [95]. E. Therapeutic activity of a single dose of pHPMA-dexamethasone conjugates (P-DEX, 10 mg/kg) on day 14, compared to 4 daily doses of free dexamethasone (DEX, 2.5 mg/kg) on the clinical arthritis score of rats with AIA. The i.v. injection of 4×2.5 mg/kg free DEX or 1×10 mg/kg P-DEX resulted in a similar strong reduction in joint inflammation. The therapeutic effect of free DEX, however, lasted only until the last injection, whereas P-DEX continued to reduce the signs of inflammation until a level similar to healthy controls [96]. F. Histological staining (H&E) of arthritic joints of rats with AIA, 10 days after treatment with P-DEX (single dose 10 mg/kg, upper-left), DEX (4 doses of 2.5 mg/kg, lower-left), saline (4 doses, lower-right), compared to healthy control (upper-right). The joints of rats treated with saline or DEX presented with clear bone destruction (single arrow) and damage of the cartilage (double arrow), and moderate synovial cell lining and villous hyperplasia (two asterisks). In the case of P-DEX treatment there was in most cases no bone and cartilage damage observed, resulting in a similar appearance as the joints of healthy rats [96].

nanoparticles prepared from poly(D,L-lactic/glycolic acid) (PLGA), poly(D,L-lactic acid) (PLA) and PEG-PLGA/PLA copolymers entrapping betamethasone disodium 21-phosphate, which is slowly released over time upon polymer hydrolysis [101–103]. Due to the sustained release kinetics of the glucocorticoid from the nanoparticles, drug concentrations could be measured in the joint up to 14 days after single intravenous administration [103]. This resulted in a long-term suppression of joint inflammation in rats with AIA, as well as in mice with collagen antibody-induced arthritis (CAIA), which in both cases was superior to a 3 times higher dose of the free drug [102].

2.3.2. Non-steroidal anti-inflammatory drugs

Due to the high risk of gastrointestinal complications, the use of non-steroidal anti-inflammatory drugs (NSAIDs) in RA therapy is currently limited [104]. However, several attempts have been made to benefit from the strong anti-inflammatory properties of NSAIDs by using a systemic drug targeting strategy. For example, indomethacin (IND), a lipophilic NSAID, has been entrapped in and conjugated to several types of nanoparticulate systems and macromolecules. IND entrapped in the bilayer of nanosized liposomes (100 nm) effectively reduced joint inflammation in adjuvant-arthritic rats, whereas a 2 times higher dose of free IND showed only a limited effect [105]. Similarly, IND encapsulated in the oily core of PEGylated long-circulating lipid nanospheres (150 nm) showed higher accumulation in joints of rats with AIA compared to free IND [106]. Although this indicates the ability of the lipid nanospheres to passively target the joint inflammation, unfortunately no therapeutic activity studies were performed. Several studies have described the application of (modified) poly(amidoamine) (PAMAM) dendrimers for the hydrophobic complexation of IND [107–109]. When complexed with 4th generation PAMAM dendrimers, 2 to 3 times higher concentrations of IND could be recovered from the joints of arthritic rats as compared to free drug administration [107]. Subsequent modifications of the PAMAM dendrimer with PEG and folate

targeting ligands were performed to further improve joint accumulation [108,109]. Surprisingly, whereas the *in vivo* anti-inflammatory efficacy of PAMAM dendrimer-IND complexes was improved compared to free IND, it was not higher than that of PAMAM dendrimers without IND, which the authors explained by an immunomodulating effect of the dendrimers themselves [110].

A polymeric methacrylamide derivative containing a 4-aminophenoxy spacer has been used to create a cleavable macromolecular delivery system for ibuprofen [111]. The hydrolytic release of ibuprofen and the 4-aminophenoxy spacer residue, which is a natural metabolite of acetaminophen (paracetamol), upon systemic injection and subsequent joint localization of the polymer-drug conjugate, resulted in an anti-inflammatory and analgesic effect *in vivo*. The selective cyclooxygenase 2 (COX-2) inhibitor celecoxib has been successfully encapsulated into albumin microspheres [112]. Although due to their relatively large size (5 μm) the celecoxib albumin microspheres mainly accumulated in the lungs, a 2.5 fold higher concentration of celecoxib was detected in the inflamed paw compared to the healthy paw of rats with mono-articular arthritis. A possible explanation might lie in the uptake of the microspheres by peripheral macrophages that subsequently traveled to the site of inflammation, taking along the microsphere-encapsulated cargo. In any case, it is evident that the targeting of NSAIDs by means of a drug targeting system is a valuable way to improve its therapeutic efficacy in the treatment of RA.

2.3.3. Methotrexate

Both liposomes and human serum albumin (HSA) have been used as carriers for arthritic joint delivery of methotrexate (MTX), a disease-modifying antirheumatic drug (DMARD) often used in RA therapy to prevent joint inflammation and disease progression [113–117]. Phospholipid-conjugated MTX incorporated into the lipid bilayer of conventional liposomes and PEG-coated, long-circulating liposomes exhibited a modest *in vivo* antirheumatic activity in rats with CIA [113,114]. Although the activity was lower than that of free MTX, the MTX liposomes were better tolerated, as indicated by a reduced hematological toxicity. In this case, the role of the liposomes probably lies more in site-specific evasion, e.g. the bone marrow, than site-specific delivery. Encouraging results for MTX delivery with respect to RA therapy were obtained with albumin-based delivery systems. When covalently coupled to HSA, 4 to 5 times lower doses of MTX were equally effective as free MTX in inhibiting the onset of arthritis in mice with CIA [115]. Interestingly, when a combination of the MTX-HSA conjugate and MTX was administered, the anti-inflammatory effect was stronger

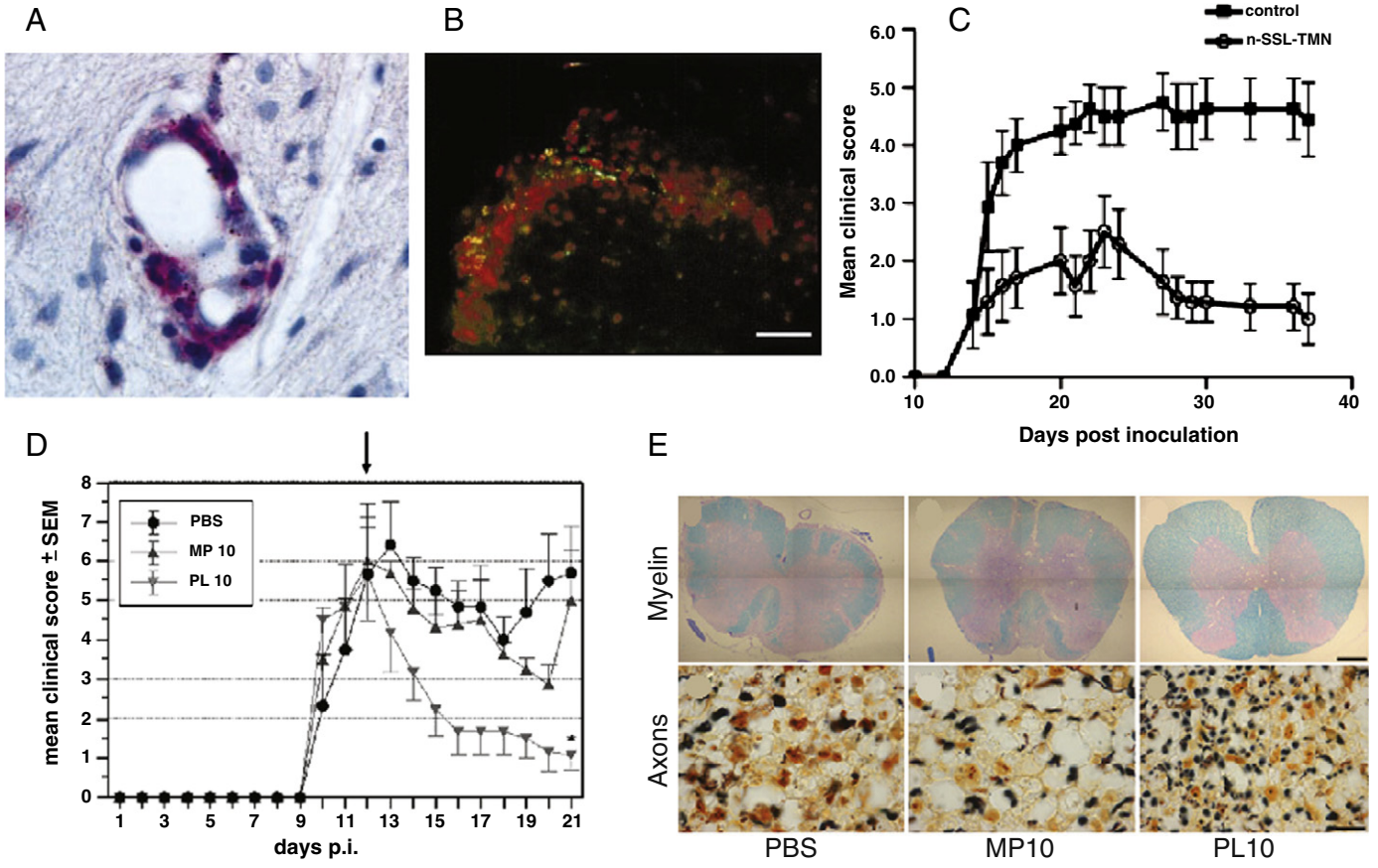

Fig. 3. Drug targeting in MS. A. Histological staining of the spinal cord of a rat with EAE showing the accumulation of gold-labeled liposomes (black dots) in relation to macrophages (ED1 mAb, red) 5 days after treatment. The liposomes were mostly located in the macrophages around the vasculature in the inflamed sites of the CNS. [28]. B. Fluorescence microscopy images of transverse sections of the spinal cord of a rat with EAE illustrating the accumulation of fluorescently labeled PEGylated polycyanoacrylate nanoparticles (green) in relation to macrophages (ED1 mAb, red) 24 h after treatment. Significant amounts of fluorescent nanoparticles accumulated in the inflamed areas in the white matter of the brain and spinal cord, which colocalized mainly in the macrophage infiltrations [27]. C. Therapeutic efficacy of tempamine-loaded PEGylated liposomes (n-SSL-TMN), upon daily i.v. injections of 8.5 mg/kg from day 10 post inoculation (p.i.), compared to saline on the clinical EAE score of mice with chronic EAE. Daily injections of liposomal tempamine, when compared to control, resulted in a significant reduction in several parameters of disease activity, such as disease duration, mean clinical score and histological score. Daily injections of free tempamine (8.5 mg/kg) did not result in significant differences compared to the control (not shown in plot) [130]. D. Therapeutic efficacy of 10 mg/kg prednisolone phosphate-loaded PEGylated liposomes (PL10), after a single injection on day 12 p.i. (arrow), compared to PBS and 3 daily injections of 10 mg/kg methylprednisolone (MP10) on the clinical EAE score of rats with acute relapsing EAE. PL10 treatment resulted in a significant alleviation of clinical systems of EAE, and protected against a relapse of disease activity. Three subsequent MP10 injections did not lead to an improvement in disease activity, and could not prevent the relapse occurring around day 20 [127]. E. Histological staining of spinal cords of rats with acute EAE, 9 days after treatment with PBS (left), MP10 (middle) or PL10 (right), illustrating the reduced demyelinization (blue, upper row) and the preservation of axons (black, lower row) upon treatment with glucocorticoid-loaded liposomes [127].

than each of them at a double dose, which indicates that MTX and MTX-HSA may act synergistically through different mechanisms [116]. Since albumin conjugates were effectively endocytosed by synovial fibroblasts and mononuclear blood cells, including monocytes, granulocytes, B cells and T cells, it is plausible that a change in cellular distribution is a main contributing factor explaining these synergistic effects [115,116]. More recently, in order to circumvent the need for exogenous albumin isolated from blood donors, a methotrexate prodrug that specifically binds albumin *in vivo* has been developed [117]. Similar to MTX-HSA, an improved therapeutic outcome of this MTX-albumin system compared to free MTX was observed in arthritic mice (CIA), confirming the potential of albumin-based drug targeting systems for RA therapy.

2.4. Drug targeting in multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), which is characterized by progressive inflammation and damage of the myelin sheet of neuronal axons in different locations within the CNS (plaques) [118]. Although initially the axon itself is preserved, the loss of myelin (demyelinization) hinders axonal conduction and will eventually lead to axonal degeneration [119–121]. As a result, MS often manifests with various neurological symptoms, including fatigue, loss of vision, diplopia, paresis and bladder dysfunction. In spite of a clear genetic predisposition and the fact that several infectious agents have been associated with the pathogenesis of MS, the underlying etiology remains unclear [118]. Since the primary target of the inflammatory response in MS is myelin, several myelin-associated proteins have been under investigation in search of the responsible antigen. Although some of these proteins, such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), are being employed to induce experimental autoimmune (or allergic) encephalomyelitis (EAE) in rodents—a condition that shows strong similarities with MS in humans and is extensively used as a preclinical model for MS—no definite antigen for MS has been identified yet [122]. During active demyelinating inflammation, activated T cells, macrophages and macrophage-like microglia infiltrate the focal plaques, attacking the myelin sheet and releasing pro-inflammatory cytokines [119,120,123]. Besides leading to axonal injury and neuronal dysfunction, the inflammatory process also disrupts the integrity of the blood–brain barrier, which normally limits the accessibility to the CNS for drugs and drug delivery systems [124]. As a consequence, drug targeting strategies employing the 'EPR-like leakiness' of the blood–brain barrier have shown promising effects in preclinical models for MS.

Glucocorticoids are commonly used in high doses to reduce disease activity in MS, like in RA, making them a good candidate for drug targeting, as targeting may help increasing the efficacy and limiting the side effects [125]. Long-circulating PEGylated liposomes containing methylprednisolone, prednisolone phosphate or dexamethasone phosphate have shown, compared to the free drug, an improved therapeutic efficacy in several studies using rat and mouse EAE models (Fig. 3D and E) [28,126–128]. In addition, liposomes encapsulating other anti-inflammatory compounds have been studied for their potency in MS. For example, minocycline, a tetracycline derivative which reduces matrix metalloproteinase 9 activity (Fig. 3C) [129], tempamine, a piperidine nitroxide which possesses anti-oxidant activity [130], and leupeptin, a tripeptide protease inhibitor [131], all have shown EAE suppressing activity upon their encapsulation into liposomes. Like in RA, there is strong evidence that the favorable therapeutic effects of targeted drug delivery systems in MS may be—at least in part—mediated by their uptake by macrophages and macrophage-like microglia, since their depletion, by using either clodronate liposomes or silica quartz microparticles, led to an alleviation of the clinical symptoms in EAE [132,133].

Interestingly, most of the work concerning drug targeting to MS has been done using liposomes [28,126–131,134–137], although there is no reason to assume that other types of drug delivery systems would be unsuitable for this purpose. Whereas there are several studies demonstrating the accumulation of liposomes in sites of active

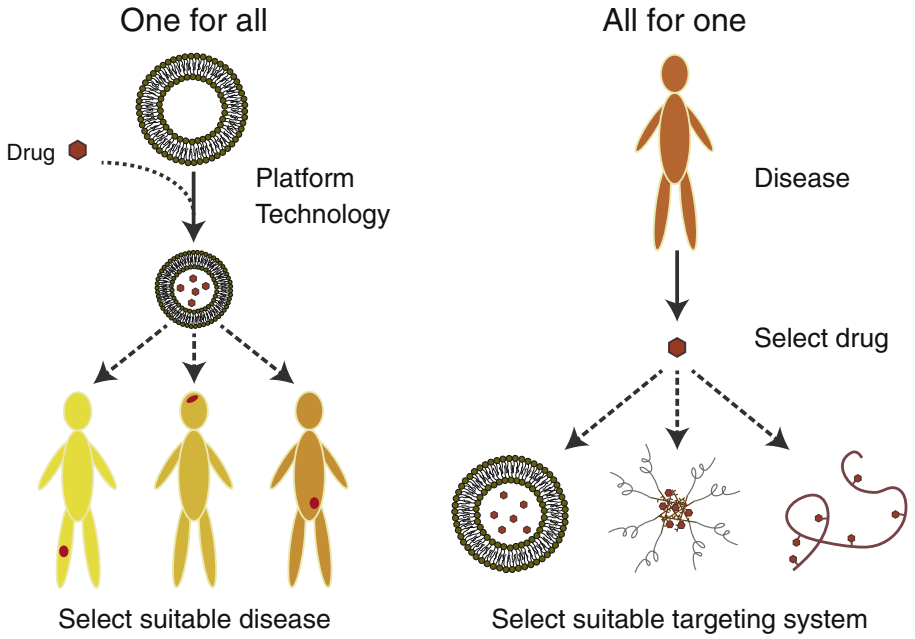

Fig. 4. Schematic representation of the dualistic approach for drug targeting research. The 'one for all' strategy focuses on a single platform technology, which may consist of a carrier system with or without a specific drug. This drug targeting system is then evaluated in various (preclinical) models of inflammatory disease, e.g. RA, MS and IBD. In contrast, the 'all for one' strategy is focused on a specific disease for which a drug is selected that could benefit of a targeted approach, e.g. due to its intrinsic low activity and/or high toxicity. For the targeted delivery of the drug, several candidate targeting systems, e.g. liposomes, micelles and polymer–drug conjugates, are then selected and evaluated in a (preclinical) model of the disease in question.

inflammation within the CNS [127,134,135], there is in fact only one report that shows the *in vivo* accumulation of a non-liposomal system, i.e. PEGylated polycyanoacrylate nanoparticles, in rats with EAE (Fig. 3A and B) [27]. However, since in the latter study only nanoparticles without a drug were used, their effectiveness in MS therapy has yet to be demonstrated.

2.5. Drug targeting in inflammatory bowel disease

Similar to RA and MS, IBD commonly presents with an intermittent course of disease, including regular exacerbations and remissions of active intestinal inflammation, and is frequently treated with glucocorticoids and other anti-inflammatory therapies [138]. Also, due to an inflammation-specific increase in intestinal vascular permeability (i.e. the EPR effect), IBD may be targeted systemically: studies using intravenously injected radiolabelled liposomes or biotinylated albumin-GdDTPA conjugates observed a 10- to 37-fold increase in accumulation of nanocarriers in inflamed colons compared to colons of healthy animals [139–141]. Whereas the EPR effect in IBD certainly enables the systemic application of passively targeted drug delivery systems, most research has focused on an oral delivery approach targeting the inflamed intestinal mucosa using e.g. polymeric micro- and nanoparticles [142–145]. For intestinal inflammatory diseases such as IBD, an oral strategy is a logical and straightforward choice, and consequently, studies employing a systemic drug delivery strategy for IBD therapy are few, and with limited success [128]. Nevertheless, the systemic nature of IBD does make systemic drug delivery a promising approach, and merits a more thorough evaluation of this strategy, especially in view of the current clinical management of IBD, for which no drug targeting system is available yet.

3. Perspectives

The research done in the context of drug delivery in inflammatory bowel disease (IBD), as described previously in Section 2.5, may be considered a good example of how drug targeting research might benefit from a more structured approach to improve its outcome. In the authors' opinion, a systematic exploration of a specific targeting technology in several preclinical models of (inflammatory) diseases on the one hand, or several targeting systems in a specific disease model on the other hand, often seems to be lacking. As discussed in this review, many inflammatory diseases, including cancer, may be targeted using the same strategic principles, e.g. using the EPR effect and upregulation of target-specific receptors, and drugs, such as anti-inflammatory and anti-angiogenic drugs. With this in mind, one could pose that it is a suboptimal use of knowledge and resources to focus all efforts on merely a single technology for targeted delivery to a single disease. Nevertheless, all too often research groups have restricted their research in this manner, focusing on the development of one technology for a specific application—in many cases a single type of cancer. As a result, there are many specialists in the field that gained extensive knowledge and experience concerning a specific delivery technology, in a specific disease, using a specific model, while in fact the role of drug targeting systems in the clinical management of these diseases remains limited. From this point of view, we wish to elaborate on a dualistic approach as schematically depicted in Fig. 4, which represents an attractive strategy for drug targeting research in order to enhance its clinically applicable.

3.1. One for all — platform technology-oriented Drug Targeting Research

The most commonly adopted strategy in drug targeting research concerns the 'one for all' approach. In this approach the emphasis is placed on a specific drug targeting technology, which is developed and optimized for drug targeting to several diseases. At a certain point in the development, the targeting system is evaluated in preclinical models, typically cancer models. Subsequent efforts are primarily focused on improving the system—often by making it more complex—and expanding the knowledge with respect to the technology.

The 'one for all' drug targeting forms a sensible and necessary element in drug delivery research: it contributes to a deeper understanding of the platform technology in question and the principles by which it works, it allows for structured patenting, and it strengthens the expertise of the research groups involved.

3.2. All for one — disease-oriented drug targeting research

In our opinion, the 'all for one' approach is advantageous in stimulating the translation of drug targeting research into clinical applications. In contrast to the technology-oriented 'one for all' perspective, i.e. taking a technology and searching for suitable applications, the disease-oriented 'all for one' perspective, which focuses on the pursuit of an optimal drug targeting system for the therapy for a specific disease, appears to be much less adopted. Based on basic knowledge concerning the underlying pathological processes, proven therapeutic efficacy *in vitro* and/or *in vivo* and current clinical treatment strategies, drug candidates which are expected to interfere with the disease are elected and applied in 'aspirant' drug targeting systems. To enable the selection of the most promising targeted carrier systems for the drug in question, there are several critical questions that should be answered. These, sometimes obvious, questions include: what are the physiochemical properties of the compound? (Is it hydrophobic or hydrophilic? What is its pKa?) What type of release kinetics is required? (Burst release? Slow release?) Which cells are the target cells? How do we reach these cells? Could a targeting ligand improve the carrier localization at these cells?

After the selection of targeted carrier candidates and their proper *in vitro* characterization, these drug targeting systems require a thorough evaluation in reliable, well-accepted clinically relevant models for the disease. Evidently, for each model the pathological pathways in which the targeted drug will be interfering should resemble the human pathology as close as possible.

The 'all for one' approach, by evaluating several drug delivery strategies utilizing the same drug in the same models, provides a better insight in which system may be optimal for that specific clinical application, which, without a doubt, improves the chances of a drug targeting system reaching clinical practice. Another important advantage of 'all for one' research is its multidisciplinary character, since only a few research groups possess sufficient expertise and experience concerning all involved targeting system technologies, *in vitro* characterization methods, and *in vivo* models for therapeutic evaluation, to successfully perform this research. Therefore, a stronger collaboration between groups, each with their own specialties regarding e.g. a drug targeting technology, *in vitro* characterization, preclinical modeling, tissue analysis, or clinical translation, is imperative. Such collaborations improve the creativity and, most likely, stimulate the generation of drug targeting systems with strong clinical potential.

4. Conclusions

Despite the evident focus on cancer therapy in drug targeting research, a large number of drug targeting systems have shown good therapeutic efficacy in various preclinical models of inflammatory diseases. Nevertheless, although many inflammatory diseases show strong similarities and may be targeted using the same principles, a thorough evaluation of one delivery system in several diseases (one for all), or reversely, several delivery systems in one disease (all for one), is often lacking. In our view, in order to stimulate the development of clinically applicable drug targeting systems, the employment

of the more systematic 'one for all' and 'all for one' approaches as proposed in this review, might prove to be highly beneficial.

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