ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 3, 2013

ISSN - 0974-2441

Review Article

NANOCARRIERS FOR TARGETING IN INFLAMMATION

JESSY SHAJI*, MARIA LAL

Department of Pharmaceutics, Prin. K. M. Kundnani College of Pharmacy 23 Jote Joy Building, Rambhau Salgaonkar Marg, Colaba, Cuffe Parade, Mumbai -05. Email: jshaji@rediffmail.com

Received: 19 May 2013, Revised and Accepted: 10 June 2013

ABSTRACT

Unselective drug availability and therefore the use of potentially too high doses are a common problem encountered today. Example for this predicament is inflammatory diseases. Side effects caused by the systemic administration of the NSAIDS and other anti-inflammatory drugs necessitate targeting of these drugs. Targeting has spatial and temporal properties which deliver the right amount of drug to the right place. Properties of inflamed barrier such as enhanced permeation and retention effect, over-expression of certain cell receptors are great targeting potentials. Tailoring with the peptide sequence of receptors facilitate active targeting. Local inflammation mediated micro environmental change can also lead to the development of stimuli responsive carriers which helps in 'smart' delivery of active pharmaceutical moieties Hence different nanocarriers like liposome, polymeric micelles, dendrimers and nanoparticles, discussed, can be targeted actively and passively for various inflammatory diseases.

Keywords: Nanocarriers, enhanced permeation and retention effect, passive targeting, active targeting, stimuli responsive carriers.

Introduction

In the last decades, targeted drug delivery has become an established field in pharmaceutical research. This concept evolved in 1906 when Ehrlich first imagined the "magic bullet" concept [1]. Inflamed tissues cells become disoriented leading to "Enhanced permeability and retention effect"(EPR) of the inflamed barrier which is an added advantage for passive targeting of the nanocarriers [2].Certain cell receptors are over expressed during inflammatory response, hence they can be used for the active targeting of the drugs . By applying these principles, drugs inflammatory diseases can be targeted at their specific sites. The challenge of the targeting is (i) to find the proper target for a particular disease; (ii) to find the drug that effectively treats this disease and (iii) to find a carrier for the drug. The specific targeting of nanocarriers leads to better profiles of pharmacokinetics and pharmacodynamics, controlled and sustained release of drugs, an improved specificity and limited off target effects. Targeting consists of "passive targeting" and "active targeting" however, the active targeting process cannot be separated from the passive because it occurs only after passive accumulation in tumors and inflamed tissues [3]

Microenvironment changes in inflammatory conditions

Targeting nanocarriers to inflammatory conditions needs an elaborate study on the microenvironment of the inflamed areas. Hence knowledge of the microenvironment is necessary to design the new targeted therapeutic systems. Based on numerous differences between normal and the inflamed tissue new avenues are open for researches to target the inflamed tissues. Angiogenesis, EPR effect, over-expression of the receptors, pH and temperature difference of the vasculature play an important role in targeting inflammation.

It is observed that under certain circumstances such as inflammation/hypoxia, tumors, infarcts, and some other pathological sites in the body, the endothelial lining of the blood vessel wall becomes more permeable than in the normal state of the tissue. As a result, large molecules and even relatively certain particles ranging from 10 to 500 nm in size can leave the vascular bed and accumulate inside the interstitial space. This was clearly demonstrated in many tumours and in infarcted areas. Assuming these large polymeric molecules/particles are loaded with an active pharmaceutical moiety, which can bring this moiety into the area with the increased vascular permeability, where the active drug can be released from a carrier. Such spontaneous accumulation or "passive" targeting, which works especially good with tumors and inflamed areas, is currently known as enhanced permeability and retention (EPR) effect [4].Maeda and colleagues first observed the EPR effect in murine solid tumors. The EPR effect has been observed in many chronic inflammatory disorders and can be used to treat such diseases as well.

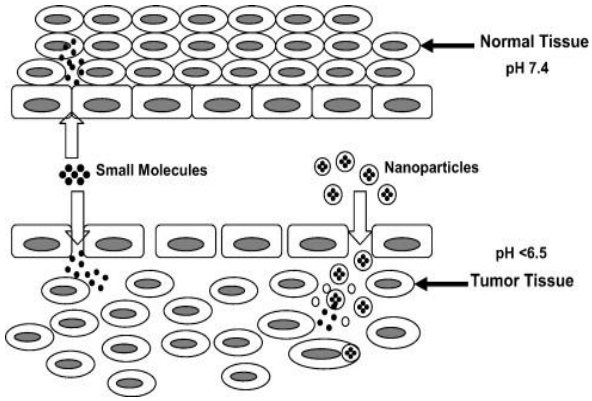

Figure 1: Passive targeting using the enhanced permeability and retention effect [5]

Structural changes in vascular pathophysiology of the inflamed vasculature could provide opportunities for the use of long-circulating particulate carrier systems. The ability of vascular endothelium to present open fenestrations was described for the sinus endothelium of the liver[6], when the endothelium is disturbed by inflammatory process, hypoxic areas of infracted myocardium [7] or in tumors [8]. To be more specific, tumor blood vessels are generally characterized by abnormalities such as high proportion of proliferating endothelial cells, pericyte deficiency and aberrant basement membrane formation leading to an enhanced vascular permeability. Particles, such as nanocarriers (in the size range of 20–200 nm), can extravasate and accumulate inside the interstitial space with endothelial pores having sizes varying from 10 to 1000 nm [9].

However, the EPR effect has not been seen particularly in tumors. In 1971, 15 years before the study of Matsumara and Maeda, Kushner

and Somerville showed 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 [10]. However the exact mechanism is unknown, one of the suggested mechanisms was an inflammation-induced 6- to 40-fold increase of blood–joint barrier permeability for high molecular weight molecules [11]

Macrophages also play a critical role in the initiation and maintenance of inflammation that leads to tissue destruction. Nanocarriers having size range of more than 200 nm are taken up by the macrophages. This is related to the opsonization process, which results in their phagocytosis after the adsorption of complement components, such as immunoglobulin G (IgG) and fibrinogen. *In vitro*, the uptake of nanoparticles by J-744 macrophages has been shown to occur via an endocytosis process, after which the particles end up in the lysosomal compartment where they are degraded; thus, affecting the delivery of the drugs to the cell cytoplasm. Hence targeting macrophages through nanocarriers can be provide great potential in inflammatory diseases such as RA where cytokines as TNF-a play a central role in inflammatory process. RA is characterized by a chronic inflammation of the joint synovium where macrophages are activated and proliferate in an abnormal way, leading to the production of proinflammatory cytokines and MMPs[12].Eventually, these factors result in the formation and activation of osteoclasts, which has disastrous consequences for the bone and cartilage. Nanoparticles could also find an application in macrophage-mediated neuro-inflammatory diseases. Due to their capacity to reach the central nervous system (CNS), circulating macrophages could act as carriers for therapeutic drug-loaded nanoparticles which can be of importance for targeting therapies to treat neurological disorders [13].

Inflammatory cells and mediators are an essential component of the tumor microenvironment. Inflammatory circuits can differ considerably in different tumors in terms of cellular and cytokine networks and molecular drivers. However, macrophages are a common and fundamental component of cancer promoting inflammation which can be thus targeted. Drivers of macrophage functional orientation include tumor cells, cancer-associated fibroblasts, T cells and B cells [14]

The pH value of pathological tissues subjected to inflammation is significantly different from the healthy tissue. It is observed that the pH is significantly less (approx 6.4) than the healthy tissue which is 7.4[15]. Drug release from the nanocarriers was shown to increase as the pH decreased in inflamed microenvironment of RA. In addition to temperature and pH, other environmental triggers may be used. For example, elevated elastace levels have been closely linked with inflammation and the increased enzymatic activity maybe be used for cleavage. The upregulation in enzymes and reactive oxygen species (ROS) may additionally serve as a means of active targeting [16].

Nanoparticles properties essential for targeting

Matsumura and Maeda showed that macromolecules in the molecular weight range of 15,000–70,000 g/mol, with certain additional properties, can effectively accumulate in a solid tumor [17]. This accumulation occurred faster with smaller molecules compared with larger, but the larger molecules were retained longer within the tumor. Thus, size is an important factor for controlling tumor accumulation kinetics and for preventing diffusion back into the systemic vascular bed. Other studies have shown that liposomes (90 nm diameter) extravasate from leaky tumor vessels but do not diffuse away effectively from the tumor even after a week [18]. The effect of nanoparticle size on blood circulation, however, is more complex and does not obey the same rules as observed for small molecule or protein-based chemotherapeutics. The impact of particle size on biodistribution has been studied using particles with wide size distribution. Particle size is known to be intrinsically related to the rate of clearance from the blood circulation, and in general smaller particles (in the range of 50–300 nm) have slower removal from the circulation compared with those having larger size [19,20]. Since long-term circulation in blood is important for targeted drug delivery and sustained release [21], studies using nanoparticles with narrow size distribution will be critical for optimizing desired biodistribution parameters. Because of the heterogeneity of tumors and dynamic status of each tumor, it will be very difficult to assume any maximum single value for particles to exploit the EPR effect in cancer related inflammation [22].

More emphasize has been given to the size and the pharmacokinetic profile of the drug carrier for passively targeted drug delivery systems [23] and [24]. 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 [25]. The long circulation time of these carriers increases the probability for target accumulation of the drug to take place. However higher amount of drug is necessary to passively accumulate the drug by the EPR mechanism , but the term 'targeted' may appear somewhat illusory in this context [26] and [27]. Macromolecules and some nanoparticulate carrier systems that are comparatively large are cleared renally from the body which are eventually taken up by phagocytic cells of the reticuloendothelial system (RES), mainly in liver and spleen [28]. 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 [29].The efficacy of the coating procedure to hinder opsonization depends on the chain length and the density of these moieties at the surface of the particles [30]. Among the most studied nanocarriers are the polyalkylcyanoacrylate (PACA) nanoparticles, which can be prepared either by polymerization of alkylcyanoacrylate monomers or directly from the polymers. The different preparation methods lead to the obtaining of nanospheres, oil- and water-containing nanocapsules and core-shell nanospheres [31]. PACA-based nanoparticles are considered to be very promising and have the capacity to bind a wide range of drugs in a non-specific manner, thus expanding their usefulness for several therapies [31]

Passive drug targeting

RA is characterized by synovial proliferation with chronic inflammatory cell infiltration and new vessel formation [32]; neoangiogenesis is essential to the survival of the synovial tissue, and it has been shown that endothelial cell turnover is higher in synovial tissue from patients with RA than from patients with osteoarthritis or healthy controls [33,34].However this effect has been mostly studied in neoplastic tissue, but its correlation with RA is quite successful. Although lymphatics tend to be better developed in rheumatoid tissue, it is well proved that the vessels show substantially enhanced permeability to macromolecules[35].This phenomenon has been applied for the therapeutic targeting of macromolecules to the synovial joints. For example, methotrexate (MTX) has a relatively short plasma half-life, due to rapid renal excretion, while conjugation to albumin prolongs its circulating half-life and improves pharmacokinetics in animal tumor and arthritis models [36, 37]. It has been observed that increased vascular permeability in the target tissues is beneficial for successful application of passively targeted drug delivery strategies in models of inflammatory diseases such as RA and MS [37], [38], [39]

Radiolabeled albumin accumulates in inflamed joints [38], and an albumin/MTX conjugate has been shown to be significantly more effective than MTX for both treatment and prophylaxis of collagen-induced arthritis, probably due to a combination of nonspecific synovial tissue homing and active uptake of albumin by synovial fibroblasts [40]. Radiolabeled human immunoglobulin has been used successfully for imaging of the inflamed synovium, with enhanced predictive value for the subsequent development of RA [41]; again this is probably due predominantly to nonspecific uptake, although interaction with specific receptors may also be a factor. Liposomes are vesicles consisting of a phospholipid bilayer encapsulating an aqueous core, and they can be used as a vehicle for a variety of therapeutic compounds [42]. It was observed that liposomes were

hampered by their rapid clearance by the reticuloendothelial system, hence limiting their use to the targeting of these organs; PEGylation and other surface modifications which reduce opsonization in the circulation can substantially increase their circulating half-life. Radiolabeled PEGliposomes have been developed to image inflamed joints [43, 44]. Encapsulation of clodronate into liposomes increases retention time in the joint and enhances uptake by macrophages due to phagocytosis of non-PEGylated liposomes, and macrophage depletion has been shown after intra-articular injection of clodronate-liposomes in patients with RA [45].

Metselaar and colleagues demonstrated that liposomal prednisolone phosphate was predominantly more efficacious than the same dose of the free drug in 2 murine arthritis models [46, 47]. They were able to show that the intravenously administered PEG-liposomes targeted to the inflamed joints showed associated reduction in cartilage damage. On histologic examination, liposomes were shown to accumulate mainly in the synovial lining layer. However, the equivalent therapeutic outcome could be possible only by repeated pulsed treatment with 10-fold higher doses of free prednisolone. Successful incorporation of the lipophilic anti-RA drug, actarit, into solid lipid nanoparticles (SLN) showed actarit-loaded SLNs exhibited sustained release after an initial burst release [48]. These results suggest that injectable SLNs may serve as passive targeting agents in RA and may offer reduced doses, decreasing dosing frequency and lowered toxicity, thereby improving patient compliance. Long-circulating, biocompatible and biodegradable polymeric nanoparticles are attractive nanocarriers for vascular drug delivery because of relatively simple manufacturing process, loading efficiency, stability in biological fluids and low toxicity. To this end, Ishihara *et al* [49] developed a series of long-circulating (PEGylated) nanoformulations of betamethasone, a synthetic glucocorticosteroid, encapsulated in U.S. FDA-approved, biocompatible and biodegradable poly(D,L-lactic/glycolic acid (PLGA) and poly(D,L-lactic acid) (PLA) polymers (size, 45–115 nm). They showed potent and prolonged anti-inflammatory activity of these nanocorticosteroids in rats and mice with experimentally induced arthritis. This was due to prolonged circulation time, passive targeting to inflamed joints and local sustained release of betamethasone. Possibly, these passively targeted novel nanomedicines could improve the therapeutic index of corticosteroids in comparison to that of pulse therapy, intra-articular injection and liposome administration.

Sethi *et al*[50] passively loaded Vasoactive intestinal peptide(VIP) onto sterically stabilised phospholipid micelles(SSM) i.e (VIP-SSM) to generate a novel, safe, long-acting immunomodulatory nanomedicine for the treatment of RA . They showed that, unlike VIP alone or empty SSM, a single subcutaneous or intravenous injection of low-dose VIP passively loaded onto SSM (VIP-SSM) preferentially localizes in the joints of mice with CIA and significantly ameliorates arthritis by modulating key cytokines, chemokines and proteinases involved in the disease process without affecting systemic arterial pressure or inducing diarrhea. Self-association of VIP with SSM confers stability to the peptide molecule and prevents its hydrolysis and inactivation in the circulation thereby prolonging its circulation time. This sterically stabilized nanoconstruct then extravasates from the hyperpermeable (leaky) post-capillary venules in inflamed joints where VIP exerts its anti-inflammatory effects.

Solid polymeric 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 are used for passive glucocorticoid targeting in RA , which is slowly released over time upon polymer hydrolysis[51]. 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. This resulted in a long-term suppression of joint inflammation in rats with antibody-induced arthritis(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 [52].

It is has been established that 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 [53]. Although this indicates the ability of the lipid nanospheres to passively target the joint inflammation, but no therapeutic activity studies were performed.

IND complex with 4th generation PAMAM dendrimers resulted in 2-3 times concentration of drug in arthritic joints as compared to free drug [54]. The selective cyclooxygenase 2 (COX-2) inhibitor celecoxib has been successfully encapsulated into albumin microspheres [55]. 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 travelled 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.

The role of macrophages in the development of multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS, has been emphasized. Apart from demyelination its pathological markers are cellular infiltration of T-cells and macrophages, blood/brain barrier (BBB) breakdown, perivascular inflammation and the loss of axons and oligodendrocytes [56]. The most favoured pathophysiological hypothesis includes T-cell-dominated autoimmune reaction in association with proinflammatory macrophages. After their activation, they secrete an increased amount of nitric oxide (NO), which has neurotoxic effects and has been involved in the permeability of the blood brain barrier (BBB)[57,58]. In a study of experimental allergic encephalomyelitis (EAE), which serves as an animal model for MS, Brosnan *et al*.[59] demonstrated that macrophage depletion can reduce the severity of the disease in a Lewis rat model. Later on, in the same animal model, liposomes containing the drug dichloromethylene diphosphonate (Cl2MDP) were used to eliminate macrophages. This treatment resulted in a complete prevention of the clinical manifestations of EAE [60]. Five years later, the same group demonstrated that Cl2MDPliposomes succeeded in eliminating the infiltrating macrophages but not the resident parenchymal microglia [61]. Therefore, even though microglia has been reported to play a major role in the development of EAE, infiltrating macrophages are needed for their activation, probably via the release of inflammatory mediator. The lifespan of human macrophages has been estimated to be between 1 and 3 months [62], hence patients needs to be treated repeatedly. Since systematic risk assessments of given nanoparticles are lacking, special attention is needed in this area before nanoparticles can be used for specific medical applications. Tempamine, a piperidine nitroxide which possesses anti-oxidant activity [63], and leupeptin, a tripeptide protease inhibitor [64], 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 [65,66]

To achieve ultra-high tissue concentrations of glucocorticoids in the inflamed target organ and to reach a much lower systemic concentration avoiding unwanted adverse effects, long-circulating PEG-coated prednisolone liposomes were developed [67]. Using an EAE rat model prednisolone liposomes were injected intravenously and prednisolone accumulation was determined after different time intervals. After 42 h, the concentration in the CNS was threefold higher compared with healthy control animals whereas spinal cord with the highest number of inflammatory lesions and BBB damage showed an accumulation 4.5- fold higher than in healthy control animals. Only in the inflamed CNS of EAE rats could an increase of prednisolone accumulation be observed and only prednisolone

liposomes clearly showed a reduction of inflammatory macrophages in the lesions. By encapsulating prednisolone higher tissue levels were achieved and the drug was delivered to the site of inflammation without a high serum concentration of the free drug.

PEG minocycline liposomes were found to ameliorate CNS autoimmune disease [68]. Beneath its conventional application in infectious diseases minocycline has been tested in animal models of neurodegeneration, CNS inflammation and traumatic brain injury [69]. Treatment with IV PEG minocycline liposomes every five days showed similar effects on the clinical signs of EAE to treatment with daily intrapertioneal injections of minocycline. Accordingly, pharmacotherapy with long-circulating PEG-coated liposomes would enable the decrease of the total minocycline amount which would again minimize the risk of potential adverse effects.

The unusual neuroinflammation targeting of dendrimers (with no targeting ligands) in the brain was explored with *in vivo* biodistribution of fluorescent-labeled neutral generation-4-polyamidoamine dendrimers (~4 nm) in a rabbit model of cerebral palsy following subarachnoid administration. These dendrimers, with no targeting ligands, were localizing in activated microglia and astrocytes (cells responsible for neuroinflammation), even in regions far moved from the site of injection, in newborn rabbits with maternal inflammation-induced cerebral palsy. This intrinsic ability of dendrimers to localize inactivated microglia and astrocytes can enable targeted delivery of therapeutics in disorders such as cerebral palsy, Alzheimer's and multiple sclerosis [70]

A PAMAM–(COOH) 46–(NAC) 18 conjugate has been prepared using a disulfide linker, that enables relatively rapid intracellular release of the drug. The FITC-labeled anionic dendrimer is rapidly taken up by microglial cells, despite the anionic surface charge. PAMAM (COOH) 46–(NAC) 18 conjugate is non-toxic even at the higher concentrations tested *in vitro*. PAMAM–(COOH) 46–(NAC)18 conjugate is a more effective anti-oxidant and anti-inflammatory agent when compared to free NAC *in vitro*. The conjugate showed significant efficacy even at the lowest dose (0.5mM NAC), where the activity was comparable or better than that of Eunice Kennedy Shriver free drug at 8mM (16× higher dosage). This suggests that dendrimer–NAC conjugates could be effective nanodevices in decreasing inflammation and injury, induced by activated microglial cells in disorders such as cerebral palsy.[71]

High mobility group box 1 (HMGB1) highly conserved, ubiquitous proteins present in the nuclei and cytoplasm of nearly all cell types, is a necessary and sufficient mediator of inflammation during sterile and infection-associated responses [72]. RAGE is a transmembrane protein and a member of the immunoglobulin superfamily. RAGE is expressed in endothelial cells, vascular smooth muscle cells, neurons and macrophages/monocytes [73]. *In-vivo* experiments indicate that HMGB1/RAGE interaction may be important in tumor formation and proliferation, because blocking RAGE and HMGB1 can decrease tumor growth and metastasis in mice [74] Inhibiting HMGB1 activity is therapeutic in arthritis, because administration of anti-HMGB1 in collagen type II induced arthritis significantly attenuated the severity of disease [75]. Blockade of RAGE–HMGB1signaling by treatment with monoclonal anti-RAGE antibodies reduced the severity of arthritis [76]. Pharmacological agents that reduce nuclear export of HMGB1 release have been shown to attenuate arthritis and inflammation in collagen-induced arthritis, by using oxaliplatin (gold sodium thiomalate, a traditional therapy for arthritis) and pituitary adenylate cyclase-activating polypeptide and by stimulation of nicotinic acetylcholine receptor [77-79].Hence targeting HMGB1is new avenue for targeted mediated therapy.

It could be hypothesized that the disruption of the intestinal barrier at ulcerated regions could allow the selective accumulation of the particulate carrier system in the desired area in inflammatory bowel disease(IBD). Besides that approach, macrophages and dendritic cells which show a strong involvement at the inflammation site in IBD can lead to an uptake of particles into these immune-related cells[80]. Lamprecht *et al* were the first to unambiguously focus on NP and their passive accumulation in inflamed intestinal areas for the development of improved drug carrier systems. Further reducing the size of drug delivery systems to micro or nanometre scale might increase colonic residence time but can also provide additional benefits for IBD therapy. A size dependent accumulation of micro- and nanoparticles (MP and NP) can be observed specifically in the inflamed intestinal regions. The effect was first described for negatively charged polystyrene particles applied orally for three days in a trinitrobenzene sulfonic acid induced (TNBS) rat model of colitis[81].In addition to the above mentioned complex nanoformulations of excipients and drugs, nanocrystalline particles by themselves may prove beneficial for IBD therapy. In this context nanocrystalline silver produced by physical vapor deposition was evaluated in a DNBS ratcolitis model[82]. The silver NP reduced the clinical activity score of the disease both after oral and intracolonic administration, the latter application route giving better results. Their anti-inflammatory activity was dose dependent, with a dose of 4 to 40 mg/kg comparable or even superior to sulfasalazine control at 100 mg/kg as quantified via histopathology and reduction of expression of IL-1β, IL-12 and TNF-α. The effect is likely to be attributed to the antibiotic effects of silver ions released from the nanocrystals. When particles of different sizes are compared one simple conclusion can be drawn; that increased retention of particles of all sizes bellow 10 μm is noticed in inflamed tissue and with further size reduction the retention effect is maximized and the clearance minimized at the size of approximately 100 nm[83]

Tacrolimus is normally used as an immune-suppressive drug to inhibit transplantation rejection. The use of tacrolimus in IBD for the treatment of refractory ulcerative colitis (UC) where corticoid treatment failed [84] is also common. To avoid significant adverse effects like nephrotoxicity after unselective absorption tacrolimus was entrapped into poly (lacticco- glycolic acid) nanoparticles before oral or rectal administration to rats suffering from experimental colitis [85]. An enhanced and selective drug penetration into the inflammation site was achieved, probably due to protection of the drug from efflux systems and mucosal metabolism. Also a threefold increase in drug penetration into inflamed tissue compared with healthy tissue could be recognized using nanoparticulate drug carriers. In a study of Meissner *et al* [86] the therapeutic effect of either tacrolimus-loaded poly(lactic-co-glycolic acid) nanoparticles or tacrolimus-loaded pH-sensitive Eudragit P-4135F nanoparticles was compared. Both nanoparticle types showed reduced adverse effects in murine dextran sulphate sodium colitis. In contrast, free drug receiving groups showed nephrotoxicity as an adverse effect. Therapeutic efficiency was not significantly different for both therapeutic approaches. This leads to the opinion that the involved mechanisms are considered far more complex.

Active drug targeting

"Active targeting" is used to describe specific interactions between drug/drug carrier and the target cells, usually through specific ligand receptor interactions [87]. Ligand–receptor interactions are possible only when the two components are in close proximity (<0.5 nm)

The term "active targeting" simply means a specific "ligand–receptor type interaction" for intracellular localization which occurs only after blood circulation and extravasation. This is why increasing blood circulation time by PEGylation (i.e., modifying the surface of nanoparticles with poly (ethylene glycol)) and/or improving the EPR effect is expected to enhance delivery to the site of action.

There are evident reasons for the design of ligand-coated long circulating drug carriers:

(a) ligand (an antibody, another protein, peptide or carbohydrate) attached to the carrier surface may increase the rate of its elimination from the blood and uptake in the liver and spleen, [88], and the presence of the sterically-protecting polymer may compensate for this effect; (b) longevity of the specific ligand-bearing nanocarrier may allow for its successful accumulation even in targets with diminished blood flow or with low concentration of the surface antigen. Early experiments attempting to combine longevity and target ability in one preparation have been performed with liposomes by simple co-incorporation of an antibody and PEG into the membrane of the same liposome. Although, in other certain circumstances (careful selection of a protective polymer/targeting moiety ratio), such approach can work, the protective polymer,

however, may create steric hindrances for target recognition with the targeting moiety [89]. To achieve better selective targeting by PEG-coated liposomes or other particulates, targeting ligands were attached to nanocarriers via the PEG spacer arm, so that the ligand is extended outside of the dense PEG brush excluding steric hindrances for its binding to the target receptors. With this in mind, potential ligands were attached to the activated distal ends of some liposome-grafted polymeric chain [90]

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 leads to pathogenesis and development of these diseases [91], [92]. Both vascular endothelial cells and monocyte-derived cells, including macrophages, are related in the angiogenic process in chronic inflammatory diseases, which makes them attractive targets for an active drug targeting approach [93]. Membrane receptors that are involved in angiogenesis signaling are upregulated, marking the cells expressing them 'inflammation-specific' and creating them as possible targets for drug delivery [94].

Theoretically, the selectivity of inflammation targeting in GI tract can be improved by attachment of various ligands at the surface of the carriers. Specific interactions with the

receptors uniformly present at large areas or only in specialized areas in the GI tract will

improve bio adhesion and absorption, capacity for endocytosis and cell localization, among them are receptor-recognizable ligands, such as lectins, toxins, viral haemagglutinins, invasins, transferrin, and vitamins (Vitamin B12, folate, riboflavin and biotin), which may improve the specificity of the delivery systems for the target cells [95]

Although several receptors are recognized as being suitable targets, primarily the folate receptor (FR) and the αvβ3 integrin have been used for active drug targeting purposes [96], [97] and [98]. It has been reported that the receptor mediating the uptake, i.e. the folate receptor, is overexpressed by several epithelial tumor cells and activated macrophages [99],[100]. Since the tissue specific expression of FR makes it an attractive target, FR-directed drug targeting makes is as an excellent strategy for active drug targeting [101], [102]. FR-expressing cancer cells and activated macrophages express distinct FR isoforms, FR-α and FR-β, respectively [103] , and much research have been focused on tumor-targeting via FR-α. 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 [104].

The αvβ3 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. The αvβ3 integrin is only expressed on the luminal surface of endothelial cells that are associated with the neovascularization process making them a specific target for anti-angiogenic therapy [105]. αvβ3 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 [106]. 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[107]. Targeting of αvβ3 has been shown to be effective for drug delivery:conjugation of doxorubicin to an RGD sequence (which binds αvβ3 with high affinity) significantly enhanced its therapeutic index in a breast carcinoma xenograft model , and an RGD peptide conjugated to a proapoptotic peptide sequence, reduced experimental arthritis in mice compared with the untargeted peptide . This approach has been successful in targeting nanoparticles for gene delivery and liposomal doxorubicin in murine tumor models [107] Arginine-glycine-aspartic acid (RGD)-labeled quantum dots, QD705 (emission maximum at 705 nm), have been used for in vivo targeting and imaging of tumor vasculature in living subjects.[108]

A lipase-labile (Sn 2) fumagillin prodrug platform coupled with a unique lipid surface-to-surface targeted delivery mechanism, termed contact-facilitated drug delivery, was used to counter the premature drug release and overcome the inherent photo-instability of fumagillin. It was shown that αvβ3-integrin targeted fumagillin prodrug nanoparticles, administered at 0.3 mg of fumagillin prodrug/kg of body weight suppress the clinical disease indices of KRN serum-mediated arthritis in a dose-dependent manner when compared to treatment with the control nanoparticles (with no drug). This study demonstrates the effectiveness of this lipase-labile prodrug nanocarrier in a relevant preclinical model that approximates human rheumatoid arthritis.[109]

Koo *et al* devised a distinct therapeutic approach to actively and selectively target a DMARNs(disease modifying anti rheumatic nanomedicine) to effector cells in inflamed joints of mice with collagen-induced arthritis (CIA), a well-established animal model of rheumatoid arthritis[110]. They developed sterically stabilized phospholipid micelles (SSM; hydrodynamic diameter, ~13 nm) composed of negatively charged 1,2-distearoylsn-glycero-3-phosphoethanolamine-(polyethylene glycol)2000 (DSPEPEG2000), a component of U.S. FDA-approved Doxil^{R} (PEGylated liposomal doxorubicin), to which camptothecin (CPT), a U.S. FDA-approved selective topoisomerase I inhibitor for cancer chemotherapy, self-associates (CPT-SSM) .The synthetic polymer provides electrostatic and steric stabilizations and a longer circulation half-life to the drug in vivo as well as functional-end groups for the attachment of targeting ligands, such as antibodies, peptides and aptamers.

Koo *etal* also found that self-association of CPT with SSM increased drug solubility and stability by 25- and 3-fold, respectively, in aqueous solution in comparison to CPT alone. Importantly, because vasoactive intestinal peptide (VIP) receptors are over expressed in activated T-lymphocytes, macrophages and proliferating synoviocytes in inflamed joints of patients with RA, Koo et al surface-modified CPT-SSM with covalently conjugated VIP (CPT-SSM-VIP), a potent, pleiotropic 28-amino acid mammalian immunomodulator belonging to the glucagon-secretin superfamily of peptides, to actively target VIP receptors over expressed on these effector cells receptors in mice with collagen induced arthritis(CIA) thereby further promoting CPT efficacy and reducing its systemic toxicity. Unlike other targeting receptors in the microcirculation, such as avb3 integrins, VIP receptors are not expressed on endothelial cells. This, in turn, evades interactions of circulating CPT-SSM-VIP with VIP receptors in the microcirculation of extra-articular organs and tissues thereby prolonging circulation time and bioavailability of these nanocarriers [110]

RGD-attached gold (Au) half-shell nanoparticles containing methotrexate (MTX) for the treatment of rheumatoid arthritis (RA) has been developed , where MTX is the most widely used disease-modifying anti-rheumatic drug (DMARD) for the treatment of RA, and RGD peptide is a targeting moiety for inflammation. Upon near-infrared (NIR) irradiation, heat is locally generated due to Au half-shells, and the drug release rate is enhanced, delivering heat and drug to the inflamed joints simultaneously. RA is a chronic inflammatory disease characterized by synovial inflammation in multiple joints within the penetration depth of NIR light. When combined with NIR irradiation, these nanoparticles containing a much smaller dosage of MTX (1/930 of MTX solution) showed greater therapeutic effects than that of conventional treatment with MTX solution in collagen-induced arthritic mice. This novel drug delivery system is a good way to maximize therapeutic efficacy and minimize dosage-related MTX side effects in the treatment of RA. Furthermore, these multifunctional nanoparticles could be applied to other DMARDs for RA or other inflammatory diseases[111].TNF-α has also been implicated in diverse range of inflammatory, infectious and malignant conditions, and its importance in inflammation is highlighted by efficacy of anti-TNF antibodies or administration of soluble TNF receptors (TNFRs) in controlling disease activity in various inflammatory conditions.[112]. Hence targeting TNF- α could be the possible new avenue for targeting inflammation.

Camptothecin, originally developed as an anti-cancer drug, has recently been proposed as a new method of controlling pannus formation and reducing cartilage degradation. To circumvent problems with solubility and stability, micelles prepared from PEG phospholipids[113] were used for drug encapsulation.

Ionic complexation of tumor necrosis factor (TNFα)-related apoptosis inducing ligand (TRAIL) conjugated to PEG (PEG-TRAIL), which bears a positive charge, with negatively charged hyaluronic acid (HA) with size ranging from 100 to 270nm, dependent upon the relative concentration of the two components. One formulation of the HA-PEG-TRAIL complex was capable of significantly reducing the secretion of proinflammatory mediators relative to PEG-TRAIL alone when administered subcutaneously to arthritic mice [114], thereby emphasizing the importance of nanoparticulate carrier systems. The HA may also serve as an active targeting

FRβ is expressed selectively by activated macrophages within the pannus tissue in RA[115]. Thus, folate may serve as an effective means of actively targeting the pannus tissue. In support of this, folate-tagged cationic and anionic poly(amidoamine) (PAMAM) dendrimers loaded with indomethacin were more effective at treating arthritic rats than indomethacin-loaded dendrimers that were folate-free[116].

Adhesion molecules, including intercellular cell-adhesion molecule-1 (ICAM-1) and E-selectin, are upregulated within the newly formed vasculature and; therefore, the endothelium serves as another possibility for active targeting in RA[117]. Preliminary studies demonstrated that a large number of PLGA-PEG nanoparticles surface modified with a peptide specific for ICAM-1 were endocytosed by vascular endothelial cells as compared to unmodified particles. A similar phenomenon was observed for polyester-based polymeric nanoparticles labeled with ligand specific for E-selectin [118]. Likewise, liposomes surface modified with the polysaccharide Sialyl Lewis X, which is known to bind selectively to E-selectin, were shown to accumulate within inflamed regions when administered intravenously to arthritic mice.

The phage display method has been used to identify specific peptides sequences that can be used for targeting tumors and other disease areas in the body [119]. One of the FDA approved targeted therapeutics is Adalimumab® antibody; a human anti-TNF IgG1 used against rheumatoid arthritis is generated by phage display technique [119].

Stimuli responsive nanocarriers for targeting

A nanocarrier system incorporated with stimuli-responsive property e.g. pH, temperature, or redox potential in case of inflammation is beneficial to overcome some of the systemic and intracellular delivery barriers [120]. Anionic liposomes containing phosphatidylethanolamine (PE) have been formulated for the delivery of antisense oligonucleotides. At low pH it allows fusion of endosomal membranes and destabilization of the endosomes which is effective in the treatment of viral infections, cancer or inflammatory diseases [121].*In vitro* and *in vivo* tests were used to demonstrate that, two pH-responsive systems, dexamethasone-HPMA conjugates (P-Dex) and dexamethasone-PEG conjugates (PEG-DEX) are more effective than free dexamethasone in regards to reducing the production of proinflammatory mediators, preventing joint damage, and targeting inflamed tissue [122]Generally hyperthermia is associated with inflammation. Indomethacin incorporated in block copolymeric nanospheres was prepared from poloxamer and poly epsilon-caprolactone (PCL). These exhibited the reversible change of size depending on the temperature and were able to reduce the damage compared with the free indomethacin when evaluated by MTT assay [123]. In another interesting work, gold nanoparticles were used to prepare shell cross-linked Pluronic® (poloxamer) micelles that exhibit a reversibly thermo sensitive swelling/shrinking behavior. This property of the micelles was caused by hydrophobic interactions of cross-linked or grafted poloxamer copolymer chains in the micelle structure with raise in temperature [124]. Magnetite nanoparticles loaded by Indomethacin (IND) were characterised as anti-inflammatory drug suitable for magnetic drug targeting. The poorly water-soluble drug IND was successfully encapsulated in polylactic acid (PLA) magnetic nanospheres (NPs) by nanoprecipitation method. The evidence of successful entrapment of IND was confirmed by FTIR and spectrophotometric measurements. The prepared magnetite–PLA–IND NPs showed the response on application of external magnetic field(i.e external stimuli) and so availability for magnetic drug targeting[125].There is also an overwhelming body of evidence that inflammatory reactions are almost ubiquitously associated with oxidative stress: the presence of a plethora of highly oxidizing compounds, such as superoxide anion, hydrogen peroxide, hypochlorite (reactive oxygen species, ROS), nitric oxide or peroxinitrite (reactive nitrogen species, RNS), is a common feature of neurodegenerative diseases, such as Parkinson's and Alzheimer's, inflammatory bowel diseases, such as Crohn's or ulcerative colitis, atherosclerosis , inflammatory lung diseases, such as asthma or fibrosis , and many others.

Nanocarriers encapsulating anti-inflammatory drugs are sensitive to the presence and concentration of oxidants; the oxidation-induced physicochemical changes (e.g., swelling or solubilization in water) allow much quicker diffusional processes of dispersed molecules (e.g., anti-inflammatory drugs), which result in their release. Eventually, the carrier materials should be eliminated (e.g., through renal excretion) in order to avoid any possible complication arising from their long-term permanence in the host body. This implies that at the end of its life cycle, the carrier should be transformed into low-molecular weight, water-soluble materials[126]. Antioxidant surface loaded liposomes might preferentially localize at inflammatory sites via redox interaction where high level of reactive oxygen species (ROS) exist. Experiments have been done to investigate the role of antioxidant as a targeting ligand on the surface of liposome employing rat granuloma air pouch model of inflammation. Conventional and antioxidant loaded diclofenac (DFS) liposomes (co-enzyme Q10 and ascorbylpalmitate) was developed for i.v. In vivo drug targeting studies showed an increase in AUC, therapeutic availability of DFS in air pouch fluid (APF) and APF/serum DFS concentration ratios from antioxidant loaded liposomes compared to conventional liposomes and drug solution. The promising result suggests the role of antioxidant as a possible ligand in drug targeting to a site where at abundant ROS exist. [127]

CONCLUSION

A lot of remarkable approaches have been developed for the targeted therapy of inflammatory diseases. Almost all systems exhibit a higher specificity in terms of delivering the drug load to the site of action. Nanocarriers show great potential for selective drug delivery to inflamed barriers. Since the exact target cells are still unknown, further investigations are necessary in order to develop specific disease-related adhesion mechanisms. Until now the therapeutic options appearing closest to clinical use are based on passive targeting approaches. Other problems are release control and industrial scale-up.

REFERENCES

1. Danhier F, Feron O, Préat V: To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 2010; 148: 135–46.
2. Bae YH: Drug targeting and tumor heterogeneity. J Control. Release 2009; 133:2–3.
3. Lamer T: Improving the efficacy of combined modality anticancer therapy using HPMA copolymer-based nanomedicine formulations. Adv Drug Deliv. Rev 2010;62(2): 203–30
4. Solid Lipid Nanoparticles for Drug Delivery: Joseph, S. and Bunjes, H In: D. Douroumis and A. Fahr, editors: Drug Delivery Strategies for Poorly Water-Soluble Drugs. UK ,Oxford: John Wiley & Sons Ltd; 2013 .p.444
5. Ganta S, Devalapally H, Shahiwala A, Amiji M.A review of stimuli-responsive nanocarriers for drug and gene delivery. J Control Release 2008;126:187–204
6. Roerdink FH, Dijkstra J, Spanjer HH , Scherphof GL .Interaction of liposomes with hepatocytes and Kupffer

cells in vivo and in vitro.Biochem. Soc. Trans. 121984:335–36
7. 7Palmer TN, Caride VJ, Caldecourt MA, Twickler J , Abdullah V. The mechanismof liposome accumulation in infarction.Biochim.Biophys.Acta1984; 797: 363–68.
8. Jain RK .Delivery of novel therapeutic agents in tumors: physiological barriersAnd strategies .J. Natl Cancer Inst.1989; 81: 570–76
9. Torchilin VP. Drug targeting, Eur. J. Pharm. Sci. 2000; 11 Suppl 2: 81–91
10. Kushner I, Somerville JA. Permeability of human synovial membrane to plasmaproteins. Relationship to molecular size and inflammation. Arthritis Rheum. 1971; 14 Suppl 5: 560–70.
11. Levick JR. Permeability of rheumatoid and normal human synovium to specificplasma proteins. Arthritis Rheum.1981; 24(12):1550–60
12. Chellata F, Merhib Y , Moreauc A, Hocine L. Therapeutic potential of Nanoparticulate systems for macrophage targeting. Biomaterials 2005;26: 7260–275
13. Merodio M,Irache JM, Eclancher F,Mirshahi M, Villarroya H .Distribution of albumin nanoparticles in animals induced with the experimental allergic encephalomyelitis. J Drug Target2000;8:289–303
14. Frances R, Will B, Mantovani A. Cancer-related inflammation: Common themes and therapeutic opportunities. Seminars in Cancer Biology 2012;22: 33–40
15. Gerweck LE, Kozin SV and SJ Stocks. The pH partition theory predicts the accumulation and toxicity of doxorubicin in normal and low-pH-adapted cells. BritishJournal of Cancer 1999;79(5/6): 838–842
16. Meers P. Enzyme-activated targeting of liposomes. Adv Drug Deliv Rev. 2001; 53(3):265-72.
17. Matsumura Y, Maeda H.A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS, Cancer Res. 1986;46: 6387–92
18. Yuan F, Leunig M, Huang SK, Berk DA, Papahad-jopoulos D, Jain RK .Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. Cancer Res. 1994; 54:3352– 56.
19. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol. Rev 2001;53 :283–318
20. Barbé C, Bartlett J, Kong L, Finnie K, Lin HQ, Larkin M, Calleja S, Bush A,Calleja G.Silica particles: a novel drug-delivery system. Adv. Mater 2004;16: 1–8
21. Guan J, He H, Yu B, Lee LJ. Polymeric nanoparticles and nanopore membranesfor controlled drug and gene delivery, in: Gonsalves K, Halberstadt C, Laurencin CT, Nair L (Eds.). Biomedical Nanostructures, Wiley-Interscience, Hoboken,NJ 2007: 115–37.
22. Bae YH, Park K. Targeted drug delivery to tumors: Myths, reality and possibility. J Control Release 2011;153: 198–205
23. Konno T, Maeda H, Iwai K, Tashiro E, Maki S, Morinaga T, Mochinaga M,Hiraoka T , Yokoyama I. Effect of arterial administration of high-molecular-weightanticancer agent SMANCS with lipid lymphographic agent on hepatoma: a preliminary report.Eur. J. Cancer Clin. Oncol.1983; 19: 1053–65.
24. Valerio –Lepiniec M, Adjadj E, Minard P, Desmadril M, Keyinteractions in neocarzinostatin. A protein of the immunoglobulin fold family.Protein Eng.2002;15: 861–69.
25. Courtice FC, The origin of lipoprotein in lymph, in: H.S. Mayersen (Ed.).Lymphand the Lymphatic System. C. C Thomas Publisher, Springfield, IL, 1963: 89–126.
26. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice.Pharmacol. Rev 2001; 53: 283–318.
27. Barbé C, Bartlett J, Kong L, Finnie K, Lin HQ, Larkin M, Calleja S, Bush A, Calleja G. Silica particles: a novel drug-delivery system. Adv. Mater. 2004; 16: 1–8.
28. Guan J, He H, Yu B, Lee LJ. Polymeric nanoparticles and nanopore membranes for controlled drug and gene delivery, in: K. Gonsalves, C. Halberstadt, C.T. Laurencin, L. Nair (Eds.). Biomedical Nanostructures, Wiley-Interscience, Hoboken,NJ 2007: 115–37.
29. Savic R , Azzam T , Eisenberg A, Maysinger D. Assessment of the integrity of poly(caprolactone)-b-poly (ethylene oxide) micelles under biological conditions: a fluorogenic-based approach. Langmuir 2006; 22: 3570–78
30. Bazile D, Prud'homme C, Bassoullet MT, Marlard M, G Spenlehauer, Veillard M. Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. J Pharm Sci1995; 84:493–98.
31. Vauthier C, Dubernet C, Fattal E, Pinto-Alphandary H, Couvreur P. Poly (alkylcyanoacrylates) as biodegradable materials for biomedical applications. Adv Drug Del Rev 2003; 55:519–48
32. Koch AE. Angiogenesis as a target in rheumatoid arthritis [review].Ann Rheum Dis 2003; 62 Suppl 2:ii60–7
33. Walsh DA, Wade M, Mapp PI, Blake DR. Focally regulate dendothelial proliferation and cell death in human synovium. Am JPathol 1998;152:691–702
34. Levick JR. Permeability of rheumatoid and normal human synovium to specific plasma proteins. Arthritis Rheum 1981; 24:1550–60.
35. Stehle G, Wunder A, Sinn H,Schrenk HH, Schutt S, Frei E, etal. Pharmacokinetics of methotrexate-albumin conjugates in tumor bearing rats. Anticancer Drugs 1997;8:835–44
36. Wunder A, Muller-Ladner U, Stelzer EH, Funk J, Neumann E ,Stehle G et al. Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis. J Immunol 2003;170:4793–801
37. Calvo P, Gouritin B, Villarroya H, Eclancher F, Giannavola C, Klein C, Andreux JP .Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat, Eur. J. Neurosci 2002;15(8): 1317–26
38. Schmidt J, Metselaar JM, Wauben MHM, Toyka KV, Storm G, Gold R, Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis, Brain 2003;126(8): 1895–1904.
39. Chacko A, Hood ED, Zern BJ, Muzykantov VR .Targeted nanocarriers for imaging and therapy of vascular inflammation. Current Opinion in Colloid & Interface Science 2011;16: 215–27
40. Fiehn C, Muller-Ladner U, Gay U ,Krienke S, Freudenberg-Konrad S , Funk J, et al. Albumin-coupled methotrexate (MTXHSA)is a new anti-arthritic drug which acts synergistically to MTX. Rheumatology (Oxford) 2004;43:1097–105
41. De Bois MH, Arndt JW, Speyer I, Pauwels EK, Breedveld FC.Technetium-99m labelled human immunoglobulin scintigraphy predicts rheumatoid arthritis in patients with arthralgia. ScandJRheumatol 1996; 25:155–8.
42. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005;4:145–60
43. Boerman OC, Oyen WJ, Storm G, Corvo ML, van Bloois L, vander Meer JW, et al. Technetium-99m labelled liposomes to image experimental arthritis. Ann Rheum Dis 1997; 56:369–73.
44. Dams ET, Oyen WJ, Boerman OC, Storm G, Laverman P, KokPJ,et al. 99mTc-PEG liposomes for the scintigraphic detection of infection and inflammation: clinical evaluation. J Nucl Med 2000; 41:622–30
45. Barrera P, Blom A, van Lent PL, van Bloois L, Beijnen JH, vanRooijen N, et al. Synovial macrophage depletion with

clodronate containing liposomes in rheumatoid arthritis. Arthritis Rheum2000;43:1951–9.

46. Metselaar JM, van den Berg WB, Holthuysen AE, WaubenMH, Storm G, van Lent PL. Liposomal targeting of glucocorticoids to synovial lining cells strongly increases therapeutic benefit in collagen type II arthritis. Ann Rheum Dis 2004; 63:348–53.
47. Metselaar JM ,Wauben MH , Wagenaar-Hilbers JP ,Boerman OC,Storm G. Complete remission of experimental arthritis by joint targeting of glucocorticoids with long-circulating liposomes. ArthritisRheum 2003; 48:2059–66.
48. YeaJ, QunWang B. Pharmaceutical Nanotechnology injectable actarit-loaded solid lipid nanoparticles as passive targeting therapeutic agents for rheumatoid arthritis. International Jour. of Pharmaceutics 2008;352(1-2): 273–79
49. Ishihara T, Kubota T, Choi T, Higaki M. Treatment of experimental arthritis with stealth-type polymeric nanoparticles encapsulating Betamethasone phosphate. J Pharmacol ExpTher 2009; 329:412-7
50. Sethi V, Rubinstein I , Onyuksel H. Vasoactive intestinal peptide (VIP)loaded sterically stabilized micelles (SSM) for improved therapy ofcollagen induced arthritis (CIA) in mice. PharmSci 2002;4(Suppl):T2036.
51. Higaki M, Ishihara T, Izumo N, Takatsu M, Mizushima Y. Treatment of experimental arthritis with poly(D, L-lactic/glycolic acid) nanoparticles encapsulating betamethasone sodium phosphate, Ann. Rheum.2005;64(8): 1132–36.
52. Ishihara T, Takahashi M, Higaki M, Mizushima Y, Mizushima T. Preparation and characterization of a nanoparticulate formulation composed of PEG-PLA and PLA as anti-inflammatory agents. Int. J. Pharm. 2010;385 (1–2) : 170–75
53. Palakurthi S, Vyas SP , Diwan PV. Biodisposition of PEG-coated lipid microspheres of indomethacin in arthritic rats, Int. J. Pharm. 2005;290 (1–2):55–62
54. Chauhan AS , Jain NK, Diwan PV, Khopade AJ. Solubility enhancement of Indomethacin with poly(amidoamine) dendrimers and targeting to inflammatory regions of arthritic rats.J. Drug Target.2004; 12 (9–10):575–83
55. Thakkar H, Sharma RK, Mishra AK, Chuttani K, Murthy RR, Albumin microspheres as carriers for the antiarthritic drug celecoxib.AAPSPharmSciTech 2004;6(1): E65–E73
56. Lucchinetti C , Bruck W, Parisi J, Scheithauer B,Rodriguez M,Lassmann H. Heterogeneity ofmultiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann. Neurol2000; 47:707–17.
57. Misko TP, Trotter JL, Cross AH. Mediation of inflammation byencephalitogenic cells: interferon gamma induction of nitric oxidesynthase and cyclooxygenase-2. J Neuroimmunol 1995; 61:195–204.
58. Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med1997; 75:165–73.
59. Brosnan CF, Bornstein MB, Bloom BR. The effects of macrophage depletion on the clinical and pathologic expression of experimental allergic encephalomyelitis. J Immunol1981;126:614–20
60. Huitinga I ,VanRooijen N , De Groot CJA, Uitdehaag BMG,Dijkstra CD. Suppression of experimental allergic encephalomyelitisin Lewis rats after elimination of macrophages. J ExpMed 1990;172:1025–33
61. Bauer J, Huitinga I, Zhao W, Lassmann H , Hickey WF , Dijkstra CD. The role of macrophages, perivascular cells, and microglialcells in the pathogenesis of experimental autoimmune encephalomyelitis.Glia1995; 15:437–46.
62. Thomas ED, Ramberg RE , Sale GE , Sparkes RS, Golde DW.Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science 1976; 192:1016–8.
63. Kizelsztein P, Ovadia H, Garbuzenko O, Sigal A, Barenholz Y, Pegylated nanoliposomes remote-loaded with the antioxidant tempamine ameliorate experimental autoimmune encephalomyelitis, J. Neuroimmunol. 2009; 213 (1–2):20–25
64. Osanai T, Nagai Y. Suppression of experimental allergic encephalomyelitis(EAE) with liposome-encapsulated protease inhibitor: Therapy through the blood–brain barrier, Neurochem. Res. 1984;9 (10): 1407–16.
65. Huitinga I, Van Rooijen N, De Groot C, Uitdehaag B, Dijkstra C, Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages, J. Exp. Med. 1990;172 (4) : 1025–33.
66. Brosnan CF, Bornstein MB, Bloom BR. The effects of macrophage depletion on the clinical and pathologic expression of experimental allergic encephalomyelitis,J. Immunol. 1981;126 (2) : 614–20.
67. Schmidt J, Metselaar JM, Wauben MHM, Tokya KV, Storm G. & Gold R .Drug targeting by long circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. Brain2003; 126:1895–1904.
68. Hu W, Metselaar J, Ben L-H, Cravens PD, Singh MP, et al. PEG Minocycline-Liposomes Ameliorate CNS Autoimmune Disease. PLoS ONE 2009 4(1): e4151
69. Yong VW,Wells J, Giuliani F,Casha S,Power C.&Metz LM. The promise of minocycline in neurology. LancetNeurol.2004: 3: 744–51
70. Dai H, Navath RS , Balakrishnan B,Raja Guru B , Mishra MK, Romero R etal.Intrinsic targeting of inflammatory cells in the brain by polyamidoamine dendrimers upon subarachnoid administration .Nanomedicine (Lond).2010; 5(9): 1317–29.
71. BingW,Navath RS, Romero R, Kannan S, Kannan R.Anti-inflammatory and anti-oxidant activity of anionic dendrimer–N-acetylcysteine conjugates in activated microglial cells.International Journal of Pharmaceutics 2009;377:159–68
72. HuanY, Kevin J.Targeting HMGB1 in inflammation. Biochimica et BiophysicaActa 2010;1799: 149–56
73. Stern D, Yan SD, Yan SF, Schmidt AM .Receptor for advanced glycation endproducts: a multiligand receptor magnifying cell stress in diverse pathologic Settings.Adv. Drug Deliv. Rev.2002;54:1615–25
74. Taguchi AD, Blood C, Del G , Canet A, Lee DC, Qu W, et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature 2000;405: 354–60.
75. Kokkola R, Li J, Sundberg E, Aveberger AC, Palmblad K, Yang H, Tracey KJ, Andersson U, Harris HE. Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. Arthritis Rheum. 2003;48: 2052–58.
76. Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, Lalla E et al RAGE and arthritis: the G82Spolymorphism amplifies the inflammatory response, Genes Immun. 2002;3:123–35.
77. Zetterstrom CK, Jiang W, Wahamaa H, Ostberg T, Aveberger AC, Schierbeck H etal.Pivotal advance: inhibition of HMGB1 nuclear translocation as a mechanism for the anti-rheumatic effects of gold sodium thiomalate.J. Leukoc. Biol. 2008; 83:31–38.
78. Abad C, Martinez C, Leceta J, Gomariz RP, Delgado M. Pituitary adenylatecyclase-activating polypeptide inhibits collagen-induced arthritis: an experimental immunomodulatory therapy. J. Immunol 2001;167:3182–89.
79. Van Maanen MA, Lebre MC, Van der Poll T,LaRosa GJ, Elbaum D, Vervoordeldonk MJ, Tak PP. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice, Arthritis Rheum. 2009;60: 114–22.
80. Ulbrich W and Lamprecht A. Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases. J. R. Soc. Interface 2010;7(1):S55-S66

81. Lamprecht A, Schafer U, Lehr CM .Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm. Res. 2001; 18: 788–793.
82. Bhol KC, Schechter PJ.Effects of nanocrystalline silver (NPI 32101) in a rat model of ulcerative colitis. Dig. Dis. Sci 2007; 52:2732–2742.
83. Hoshino H ,Goto H, Sugiyama S,Hayakawa T& Ozawa T. Effects of FK506 on an experimental model of colitis in rats. Aliment. Pharmacol. Ther. 1995;9: 301–307.
84. Matsuhashi N et al. 2000 Tacrolimus in corticosteroid resistant ulcerative colitis. J. Gastroenterol. 35, 635–640.
85. Lamprecht A, Yamamoto H, Takeuchi H & KawaShima Y.Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis inrats. J. Pharmacol.Exp.Ther2005;315:196–202.
86. Meissner Y, Pellequer Y, &Lamprecht A. Nanoparticlesin inflammatory bowel disease: particle targeting versus pH-sensitive delivery. Int. J. Pharm 2006;316: 138–143.
87. Beduneau A, Saulnier P, Hindre F, Clavreul A, Leroux JC, Benoit JP. Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab'fragments.Biomaterials 2007;28: 4978–4990
88. Klibanov AL, Antibody-mediated targeting of PEG-coated liposomes, in: Woodle MC, Storm G (Eds.). Long-circulating Liposomes: Old Drugs,New Therapeutics,SpringerVerlag, 1998: pg 269
89. Benhar I, Padlan EA, Jung SH, Lee B, Pastan I. Rapid humanization of the Fv of monoclonal antibody B3 by using framework exchange of the recombinantimmunotoxinB3(Fv)-PE38. Proc. Natl. Acad. Sci. U. S. 1994;91: 12051–12055
90. Torchilin VP, Levchenko TS, Lukyanov AN, Khaw BA, Klibanov AL, Rammohan R, Samokhin GP, Whiteman KR .p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochim.Biophys. Acta2001; 1511: 397–411.
91. Costa C, Incio J ,Soares R.Angiogenesis and chronic inflammation: cause or consequence? Angiogenesis 2007; 10(3):149–66.
92. Szekanecz Z, Besenyei T,Szentpétery A, Koch AE. Angiogenesis and vasculogenesis in rheumatoid arthritis. Curr.Opin. Rheumatol.2010;22(3): 299–306
93. Kinne RW, Stuhlmüller B, Burmester GR. Cells of the synovium in rheumatoid arthritis. Macrophages. Arthritis Res. Ther 2007; 9(6):224-16
94. Cox D, Brennan M, Moran N. Integrins as therapeutic targets: lessons andOpportunities.Nat. Rev. Drug Discov. 2010;9(10): 804–20.
95. Goracinova K, Dodov M, Crcarevska Mand Geskovsk N.Drug Targeting in IBD Treatment – Existing and New Approaches, Inflammatory Bowel Disease - Advances in Pathogenesis and Management, Dr. Sami Karoui (Ed.) 2012 ISBN: 978-953-307-891-5
96. Gerlag D, Borges E, Tak P, Ellerby HM, Bredesen D, Pasqualini R, Ruoslahti E,Firestein G. Suppression of murine collagen-induced arthritis by targeted apoptosis of synovial neovasculature. Arthritis Res 2001;3(6): 357–61.
97. Low PS, Henne WA, Doorneweerd DD. Discovery and development of folicacid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases.Acc. Chem. Res 2008;41(1): 120–29.
98. Koning GA, Schiffelers RM, Wauben MHM, Kok RJ, Mastrobattista E, Molema G, et al.. Targeting of angiogenic endothelial cells at sites of inflammation by dexamethasone phosphate-containing RGD peptide liposomes inhibits experimental arthritis. Arthritis Rheum 2006;54(4): 1198–1208
99. Weitman SD, Lark RH, Coney LR, Fort DW, Frasca V, Zurawski VR ,Kamen BA. Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. Cancer Res.1992;52(12): 3396–3401.
100. Nakashima-Matsushita N, Homma T, Yu S, Matsuda T, Sunahara N, Nakamura T, et al. Selective expression of folate receptor Î² and its possible role in methotrexate transport in synovial macrophages from patients with rheumatoid arthritis. Arthritis Rheum 1999; 42(8): 1609–16.
101. Turk MJ, Breur GJ, Widmer WR, Paulos CM, Xu LC, Grote LA, Low PS.Folate- targeted imaging of activated macrophages in rats with Adjuvant induced Arthritis.Arthritis Rheum. 2002;46(7):1947–55103
102. Low PS, Antony AC. Folate receptor-targeted drugs for cancer and inflammatory diseases. Adv Drug Deliv. Rev 2004; 56(8): 1055–58.
103. 103.Wang X, Shen F, Freisheim JH, Gentry LE, Ratnam M. Differential Stereospecificities and affinities of folate receptor isoforms for folate compounds And antifolates.Biochem. Pharmacol 1992; 44(9):1898–190.
104. 104. Turk MJ, Waters DJ, Low PS. Folate-conjugated liposomes preferentially target macrophages associated with ovarian carcinoma. Cancer Lett 2004;213(2):165–172.
105. Brooks PC, Clark RAF, Cheresh DA. Requirement of vascular integrin αvβ3 for Angiogenesis. Science 1994; 264 (5158) :569–71
106. Crielaard JB , Lammers T , Raymond M , Storm G.Drug targeting systems for inflammatory disease: One for all, all for one. J Control Release 2012;161:225–34
107. 107. Garrood T and Pitzalis C . Targeting the Inflamed Synovium: The Quest For Specificity. Arthritis& Rheumatism 2006; 54(4):1055–60
108. Cai W, Shin DW, Chen K, Gheysens O , Cao Q, Wang SX , et al.Peptide-labeled near-Infrared quantum dots for imaging tumor vasculature in living subjects. NanoLett2006; 6:669-76.
109. 109.Zhou HF, Yan H,Senpan A, Wickline SA, Pan D, Lanza GM .Pham Suppression of inflammation in a mouse model of rheumatoid arthritis using targeted lipase-labile fumagillin prodrug nanoparticles. Biomaterials2012; 33(33):8632-40
110. Koo OM, Rubinstein I , ÚnyÏksel H. Actively targeted low-dose camptothecin as a safe, long-acting, disease-modifying nanomedicine for rheumatoid arthritis. Pharm Res 2011; 28:776-87.
111. 111.Lee SM, Kim HJ, Ha YJ,Park YN, Lee SK, Park YB, Yoo KH Targeted Chemo-Photothermal Treatments of Rheumatoid Arthritis Using Gold Half-Shell Multifunctional Nanoparticles.ACS Nano. 2012; 50–57.
112. Patil P, Patil L, Kadam V. TNF-α: a potential therapeutic target for inflammatory bowel disease.Asian J Pharm Clin Res 2011;4 Suppl 1: 158-166
113. Ashok B, Arleth L, Hjelm RP, Rubinstein I,Onyuksel H. In vitro characterization of PEGylated phospholipid micelles for improved drug solubilization:effects of PEG chain length and PC incorporation. J Pharm Sci.2004;93(10):2476-87.
114. Kim YJ, Chae SY, Jin CH,Sivasubramanian M, Son S, Choi KY, et al. Ionic complex systems based on hyaluronic acid and PEGylated TNF-related Apoptosis inducing ligand for treatment of rheumatoid arthritis. Biomaterials2010;31, 34: 9057- 64.
115. Nagayoshi R , Nagai T, Matsushita K, Sato K,Sunahara N,Matsuda T, et al.Effectiveness of anti-folate receptor beta antibody conjugated with truncated Pseudomonas exotoxin in the targeting of rheumatoid arthritis synovial macrophages.Arthritis Rheum 2005;52(9) : 2666-75.
116. Chandrasekar D, Sistla R, Ahmad FG,Khar RK, &Diwan PV. The Development of folate-PAMAM dendrimer conjugates for targeted delivery of anti-arthritic drugs and their pharmacokinetics and biodistribution in arthritic rats.Biomaterials 2007;28(3)504-12.
117. Banquy X, Leclair G, Rabanel JM, Argaw A , Bouchard JF, Hildgen P, et al. Selectins ligand decorated drug carriers for activated endothelial cell targeting.BioconjugChem2008;19(10) : 2030-39.

118. Hirai M, Minematsu H, Kondo N, Oie K, Igarashi K, Yamazaki N. Accumulation of liposome with Sialyl Lewis X to inflammation and tumor region: application to in vivo bio-imaging. Biochem Biophys Res Commun, 2005; 353(3):553-58.
119. Sergeeva A, Kolonin MG, Molldrem JJ, Pasqualini R, Arap W.Display technologies: application for the discovery of drug and gene delivery agents.Adv. Drug Deliv Rev. 2006; 58 (15) :1622–54
120. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysisof rare harmful effects in randomized controlled trials. JAMA 2006; 295:2275–85
121. Fattal E, Couvreur P, Dubernet C. "Smart" delivery of antisense oligonucleotides by anionic pH-sensitive liposomes, Adv. Drug Deliv. Rev.2004; 56(7):931–46.
122. Liu X, Quan M, Tian LDJ, Laquer CP, Ciborowski P,Wang D. Syntheses of click PEG dexamethasone conjugates for the treatment of rheumatoid arthritis. Biomacromolecules 2010 ;11(10): 2621-28.
123. Kim SY, Ha JC, Lee YM .Poly(ethylene-oxide)-poly(propylene oxide)poly(ethyleneoxide)/poly(epsilon-caprolactone)(PCL) amphiphilic block copolymeric nanospheres. II. Thermo-responsive drug release behaviors. J. Control. Release 2000;65: 345–58.
124. Bae KH, Choi SH, Park SY, Lee Y, Park TG.Thermosensitive pluronic Micelles stabilized by shell cross-linking with gold nanoparticles, Langmuir 2006; 22: 6380–663
125. Timko M, Koneracka M, Tomasovicova N ,Kopcansky P, Zavisova V. Magnetite polymer nanospheres loaded by Indomethacin for anti-inflammatory therapy. Journal of Magnetism and Magnetic Materials 2006; 300: e191–e194
126. Vitaliy KV, Tirelli N.Oxidation- responsiveness of nanomaterials for targeting inflammatory reactions.Pure Appl. Chem 2008;80(8);1703–18.
127. Jukanti R, Devaraj G, Devaraj R, Apte S.Drug targeting to inflammation: Studies on antioxidant surface loaded diclofenac liposomes. International Journal of Pharmaceutics 2011; 414:179– 185