REVIEW

Polymer-Drug Conjugates in Inflammation Treatment

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Summary

Inflammation is a vital defense mechanism of living organisms. However, persistent and chronic inflammation may lead to severe pathological processes and evolve into various chronic inflammatory diseases (CID), e.g. rheumatoid arthritis, multiple sclerosis, multiple sclerosis, systemic lupus erythematosus or inflammatory bowel diseases, or certain types of cancer. Their current treatment usually does not lead to complete remission. The application of nanotherapeutics may significantly improve CID treatment, since their accumulation in inflamed tissues has been described and is referred to as extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration (ELVIS). Among nanotherapeutics, water-soluble polymer-drug conjugates may be highly advantageous in CID treatment due to the possibility of their passive and active targeting to the inflammation site and controlled release of active agents once there. The polymer-drug conjugate consists of a hydrophilic biocompatible polymer backbone along which the drug molecules are covalently attached *via* a biodegradable linker that enables controlled drug release. Their active targeting or bio-imaging can be achieved by introducing the cell-specific targeting moiety or imaging agents into the polymer conjugate. Here, we review the relationship between polymer conjugates and inflammation, including the benefits of the application of polymer conjugates in inflammation treatment, the anti-inflammatory activity of polymer drug conjugates and potential polymer-promoted inflammation and immunogenicity.

Key words

Inflammation • Polymer conjugates • Anti-inflammatory nanotherapeutics • Immunogenicity

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Introduction

The past few decades have witnessed an exponential growth in nanotechnology-based therapeutic approaches, including numerous nanosized drug delivery systems or diagnostics, being developed to improve treatment or diagnosis of various diseases. Since cancer is one of the main causes of death worldwide, the enhancement of anticancer treatment has been the major driving force behind nanomedicine design. Nevertheless, chronic inflammatory diseases (CID), e.g. rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus or inflammatory bowel diseases, have a substantial socio-economic impact, since their prevalence is very high. The current therapy can reduce disease symptoms, though it cannot ensure a complete cure and patient quality of life is very limited, involving chronic pain states, surgical interventions and incapacity for work (El-Gabalawy *et al.* 2010). In addition, persistent chronic inflammation is considered as a potential risk factor in the development of various severe diseases, including cancer, diabetes, atherosclerosis or Alzheimer's and Parkinson's disease (Chen *et al.* 2018).

Remarkably, limited attention has been paid to the development of inflammation-targeted therapy based on nanomedicines, although many strategies utilized in tumor-targeted drug delivery could be applied for drug delivery to inflammation sites (Coussens and Werb

2002). With an increasing number of clinical trials and approvals of nanotechnology-based application in medicine, it is essential to investigate their detailed impact on human physiology and pathophysiology and to evaluate their potential immunogenicity and pro-inflammatory effect.

The common denominator of nanosized systems is their size, which is generally <100 nm in at least one dimension. However, their chemical structure, behavior in biological fluids and thus their interaction with biological systems at the molecular, cellular or organ level may be diametrically different. Nanosized drug carriers can considerably prolong drug circulation in the blood, improve their pharmacokinetics and minimize drug side effects (Ikoba *et al.* 2015). Among nanomedicines, water-soluble polymer-drug conjugates may be highly beneficial in CID treatment due to their potential passive and active targeting to the inflammation site and the controlled release of active agents once there. Generally, the polymer conjugates consist of a water-soluble polymer backbone along which various drug molecules are attached *via* biodegradable spacers (Kostka and Etrych 2016, Lidicky *et al.* 2016, Pola *et al.* 2016). Moreover, targeting moieties or imaging agents may be introduced to the polymer structure. Compared to widely used nanoparticles and liposomes with physically loaded drugs, water-soluble polymer conjugates exhibit lower immunogenicity and stimuli-responsive release of biologically active compounds (Natfji *et al.* 2017).

Here, we review the relationship between polymer conjugates and inflammation, namely the benefits of the application of polymer conjugates to inflammation treatment, the anti-inflammatory activity of polymer-drug conjugates and potential polymer-promoted inflammation and immunogenicity.

Inflammation

Inflammation is immune system defense mechanism vital for removing harmful stimuli that arise during infection or injury and initiating the healing process. However, dysregulation of the immune response and inadequate resolution may lead to chronic inflammation with severe clinical symptoms (Headland and Norling 2015). Due to the complexity of inflammation process and its resolution, the detailed description of inflammation mechanisms is out of scope of this article and it has been recently reviewed (Ashley *et al.* 2012, Sugimoto *et al.* 2016).

Mechanisms of acute and chronic inflammation

Inflammation is a strictly regulated cascade of immunological, physiological and behavioral processes. The first step is the recognition of the source of damage or infection. Pathogens are identified by their pathogen-associated molecular patterns (PAMPs). Damage-associated molecular patterns (DAMPs) are released by cells in reaction to stress, injury or necrosis (Bianchi 2007). In addition to pathogens, biomaterials, including some polymer materials, may also be recognized as foreign bodies and the immune reaction is initiated by protein interactions at their surface. Albumin, fibronectin or fibrinogen adsorb on biomaterials, which results in the recruitment of neutrophils and activation of resident mast cells, which represents the acute inflammatory response (Anderson *et al.* 2008).

Damage signals, based on PAMPs and DAMPs, are further recognized by transmembrane Toll-like receptors and intracellular binding domains and leucine-rich-repeat-containing receptors. The subsequent cascade results in NF-κB activation and further expression of proinflammatory cytokines such as interleukins IL-1β and IL-6, interferon-γ or tumor necrosis factor alpha (TNFα) (Janssens and Beyaert 2003). These effectors attract immune cells such as neutrophils and monocytes. Activated neutrophils trigger cascades, which leads to bacterial killing and the release of reactive oxygen species (ROS) and proteinases into the extracellular space and the destruction of pathogens as well as host tissue (Wright *et al.* 2010). All these cascades lead to clinical signs of inflammation: heat, swelling, redness, pain and loss of function.

The last step of inflammation is its resolution. After neutrophils fulfill their function, an apoptotic pathway is triggered by cytokines produced by macrophages (Van Den Berg *et al.* 2001). Apoptotic neutrophils promote their own clearance by expressing find-me and eat-me signals, allowing the identification of a dying cell, and produce mediators that inhibit further neutrophils recruitment (Elliott *et al.* 2009).

Macrophages clear the neutrophils through so-called efferocytosis. Macrophages undergo a transition in phenotype from pro-inflammatory to pro-resolution macrophages that produce anti-inflammatory factors such as IL-10 or TGF-β (Fadok *et al.* 1998). Resolution-phase macrophages secrete T- and B-lymphocytes chemoattractants and factors, which helps the recovery of tissue homeostasis (Rajakariar *et al.* 2008). More blood monocytes are recruited to the site where differentiation

into pro-resolution macrophages is completed (Melnicoff *et al.* 1989).

When this balanced immune cascade fails, acute inflammation develops into chronic one. The initial cause of the reaction (bacteria, injury) is no longer present and immune cells attack host tissue and cause damage. In the case of chronic inflammation, neutrophils do not undergo apoptosis as intended and transform into long-living cells and continue producing ROS and proteases and damage the surrounding tissue. Without adequate signals from apoptotic neutrophils, macrophages do not change their phenotype towards pro-resolution. Inflammatory cells produce more pro-inflammatory cytokines and chemokines, which results in the production of oxygen and nitrogen reactive species (Fox *et al.* 2010). Chronic inflammation is often age-related and contributes to age-associated diseases (Franceschi and Campisi 2014).

Chronic inflammatory diseases and inflammation-related diseases

While acute inflammation is a normal host response to pathogen infections and tissue injury, its inadequate resolution can lead to the chronic stage, which is the precursor to a variety of diseases, e.g. CID or cancer. Although the clinical symptoms of diverse CID are distinctly different, the cellular processes in chronically inflamed tissues are similar, involving leukocyte migration, vasodilatation, activation of endothelial adhesive molecules, fluid extravasation, cellular influx and eventually angiogenesis or tissue fibrosis leading to the loss of tissue function (Bodolay *et al.* 2002). The current CID therapy includes the use of glucocorticoids (GC), non-steroidal anti-inflammatory drugs (NSAID) and immuno-modulating agents, since these diseases are often connected with autoimmunity. The drugs used alone or in combination suppress some symptoms and their use leads to partial treatment efficacy, but complete disease remission is very rare. The long-term administration of these agents causes severe side effects, e.g. nephrotoxicity, gastric ulceration, osteoporosis or hematotoxicity (Dinarello 2010).

Rheumatoid arthritis (RA). In RA, the primary site of inflammation is the synovial tissue in diverse joints. This chronic autoimmune inflammatory disease causes progressive articular damage, functional loss, and comorbidity, e.g. insulin resistance, dyslipidemia and coronary heart diseases (McInnes 2001).

Multiple sclerosis (MS). MS is an inflammatory demyelinating disease of the central nervous system.

Permanent disability is caused by the loss of axonal function, which correlates with the degree of inflammation (Bruck and Stadelmann 2003).

Inflammatory bowel diseases (IBD). IBD including ulcerative colitis and Crohn's disease describe conditions with chronic or recurring inflammation of the gastrointestinal (GI) tract, especially the large intestine, leading to blood- and mucus-containing diarrhea, severe painful states or GI tissue damage potentially requiring surgical intervention (Kaser *et al.* 2010).

In addition, persistent chronic inflammation is considered a potential risk factor in the development of other severe diseases, including cancer, atherosclerosis, diabetes or Alzheimer's and Parkinson's disease (Chen *et al.* 2018).

Cancer. Chronic inflammation is an important factor for cancer development, causing 1 in 4 cancer cases, according to epidemiological data. Inflammation induces DNA damage and chromosomal instability, which enhances tumor cell proliferation and resistance to apoptosis (Hussain and Harris 2007). Tumor metastasis is promoted by angiogenesis, a standard process accompanying inflammation (Mantovani *et al.* 2008).

Polymer conjugates

Polymer conjugates (PCs), also known as macromolecular prodrugs, are high molecular weight (HMW) conjugates, typically carrying several molecules of low-molecular-weight drugs or their combination bound to a polymer backbone designed to optimize drug pharmacokinetics (Fig. 1). PCs have a molecular weight (M_w) significantly exceeding that of the conjugated drug and their higher hydrodynamic radius decelerates renal excretion of PCs and thus prolongs the circulation of the drug in the blood stream. In addition, PCs generally cannot penetrate healthy endothelium, which minimizes the delivery of conjugated drugs to non-targeted tissues and organs. However, the endothelium of inflamed tissues is more permeable for macromolecules compared to healthy tissues, thus enabling the passive accumulation of PCs. The drug is usually inactive when conjugated to the polymer backbone. The biodegradable spacer between the drug and the polymer chain based on enzymatically or reductively cleavable linkers or hydrolysable bonds enables controlled drug release in the target tissue or cells. Passive accumulation, controlled drug release and the potential introduction of targeting moieties to specific cells or imaging agents make PC with anti-inflammatory

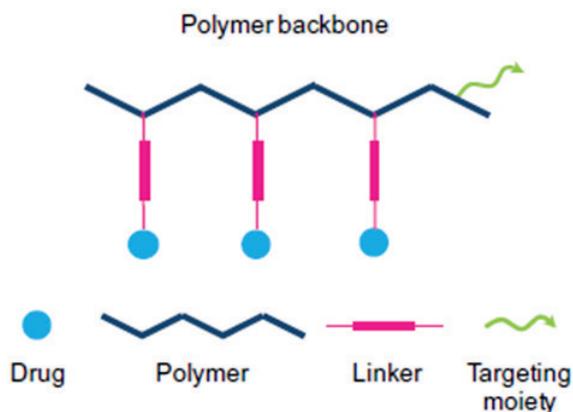drugs promising candidates for problematic CID treatment (Natfji *et al.* 2017).

Polymer backbone

Fig. 1. The scheme of the polymer conjugate with the drug and targeting moiety (modified from (Natfji *et al.* 2017)).

Polymer backbone type

The key components of successful PCs are the polymer backbone and the linkage between the drug and the carrier. The polymer backbone of PCs typically serves only as a drug carrier and has no inherent beneficial pharmacological activity. Nevertheless, the "stealth" property of the polymer carrier is its most appreciated feature, improving drug pharmacokinetics and reducing its side effects.

Despite their different origin, i.e. synthetic or natural, and diverse chemical structure, the polymers used in PC design should be water-soluble, biocompatible, non-toxic and non-immunogenic, with functional moieties available for the attachment of drugs, targeting molecules or imaging agents.

The polymer backbone can be biodegradable or non-biodegradable. Water-soluble biodegradable polymers utilized for PCs, which are based on e.g. poly(L-lysine) (Ryser and Shen 1978), poly(L-glutamic acid) (Yang *et al.* 2011), dextrins (Baldwin and Kiick 2010), chitosan (Onishi *et al.* 2011) or hyaluronic acid (Shin *et al.* 2014), are degraded to small fragments, thereby facilitating their excretion from the organism. Frequently, biodegradable natural polymers have to be modified to be able to serve as drug carriers. In spite of their undeniable benefit of biodegradability, some of them become toxic and immunogenic when administered to the body (Markovsky *et al.* 2012).

Non-biodegradable polymers are not subject to biodegradation and are usually excreted by the kidneys *via* glomerular filtration up to a certain limit of their M_w,

e.g. for the vinylic type of polymers, the limit is approximately 50 000 g/mol. The polymers of higher M_w are eliminated very slowly *via* the hepatobiliary route or accumulate in the organism (Markovsky *et al.* 2012). Despite their size limitation, several benefits of non-degradable polymers frequently used in drug delivery, e.g. non-toxicity, non-immunogenicity, system versatility or potential introduction of various functional groups, outweigh their non-biodegradability. Various water-soluble polymer-drug conjugates are based on numerous non-degradable polymers, e.g. poly(ethylene glycol) (PEG) (Liu *et al.* 2010), copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) (Liu *et al.* 2008), poly(vinyl *N*-pyrrolidone) (Timofeevski *et al.* 1996) or poly(2-oxazoline) (Luxenhofer *et al.* 2012).

The synthetic polymers are highly attractive in PC design, since they offer countless means for conjugation with biologically active compounds, including reactions in organic solvents or the multivalence of some synthetic polymer precursors. Polymers based on HPMA have been particularly thoroughly investigated in the field of PCs (Kopeček and Kopečková 2010, Kopeček 2013). The high interest in HPMA-based polymers has been aroused by their numerous clear benefits, i.e. biocompatibility and non-immunogenicity, high hydrophicility, which improves the solubility of conjugated drugs, the possibility of introducing numerous different functional groups or the potential co-polymerization of the drug-containing monomer. In addition, the progress in controlled polymerization techniques, especially controlled radical reversible addition and fragmentation transfer (RAFT) polymerization, has enabled the synthesis of various well-defined HPMA-based polymers with a narrow distribution of molecular weights (Chytil *et al.* 2018).

In addition to HPMA-based polymers, PEG has largely been used in PCs to prolong blood circulation of low molecular weight (LMW) drugs and proteins. Although several PEGylated therapeutically active proteins have been introduced into clinical practice, no PEG conjugate with LMW drugs has successfully been marketed despite countless publications concerning this topic (Pasut and Veronese 2009). This phenomena can be ascribed to PEG limitations, especially its scarcity of conjugation sites and poor options for copolymerization, allowing only one drug molecule per linear PEG chain (Harris and Chess 2003). In order to overcome this disadvantage, PEG-based dendrimers and block

copolymers enabling the conjugation of more than one drug per chain have been synthesized (Zacchigna *et al.* 2011). However, safe excretability should be taken into consideration during the design of the complex PEG-based structures, since the PEG chain is non-biodegradable and its renal threshold is approximately 30 kDa (Baumann *et al.* 2014). In addition, the widely accepted "golden standard" position of PEG in bioapplication should be carefully reconsidered, since its claimed anti-immunogenicity has lately been disproved (see chapter 4).

The majority of polymer backbones for PCs are charge-neutral, since polycations and polyanions are believed to be notoriously toxic through diverse mechanisms, to accumulate in various organs through nonspecific interactions and to induce significant systemic side effects such as induction of interferon production (Merigan and Regelson 1967, Moreau *et al.* 2002).

A specific group of polymer precursors for PCs are dendrimers and hyperbranched polymers, which are three-dimensional, highly ordered star-like oligomeric and polymeric macromolecules with numerous functional groups on the surface. The major advantage of using dendrimers for biomedical applications is their monodispersed chemical structure as well as abundance of surface functional groups, which enables a high drug conjugation rate. The physiochemical properties, e.g. solubility, stability, functionality and charge density, are determined by the chemical structure of the monomer unit(s) in the core and surface. The most frequently used dendrimers in drug delivery are polyamidoamine (PAMAM) dendrimers, despite the poor biocompatibility of PAMAM dendrimers with non-modified surface amino groups and their difficult and costly synthesis (Svenson and Tomalia 2005). Hyperbranched polymers and dendrimers based on polyglycerols exhibit good biocompatibility and non-immunogenicity; in addition, their synthesis is relative simple and scalable (Abbina *et al.* 2017).

A suitable biodegradable linker between the polymer backbone and drug is often a prerequisite for successful treatment. Due to the acidity of the inflammation site, several anti-inflammatory PCs employ a pH-sensitive hydrazone bond. In addition, the presence or higher concentration of specific enzymes in inflamed tissue has led to the design of enzymatically cleavable spacers. PCs intended for CID treatment are described in detail in chapter *Polymer conjugates with anti-inflammatory activity*.

Targeting of polymer conjugates to the inflammation site

The principle of passive targeting of PCs is similar to that of tumor-targeted nanomedicines, known as the enhanced permeability and retention (EPR) effect in solid tumors (Maeda and Matsumura 1989). The endothelium of inflamed tissues, e.g. in arthritic joints or the blood-brain barrier during MS, is more permeable for macromolecules compared to healthy tissues and the rate of accumulation in inflamed tissues is molecular-weight dependent (Kushner and Somerville 1971, Kwon and Prineas 1994, Bennett *et al.* 2010). In the RA animal model, it was shown that the leaky vasculature of inflamed joints enables permeation by nanomedicines, which are subsequently internalized by activated synoviocytes exhibiting an enhanced endocytic pathway. This phenomenon is referred to as extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration (ELVIS). ELVIS may also probably play a role in other CID (Yuan *et al.* 2012). The shape, size and M_w of polymer therapeutics are the key factors for their biodistribution and pharmacokinetic profile. With the growing size of polymer carriers, their blood circulation is prolonged and their accumulation in inflamed tissues increases up to a certain limit. Nevertheless, the water-soluble non-biodegradable polymer carriers with a M_w above 50,000 g/mol cannot be excreted by the kidneys and therefore HMW systems should degrade to easily excretable fragments to prevent their persistence in the body (Quan *et al.* 2010).

The ability of various polymer carriers to accumulate at the inflammation site has been repeatedly shown using diverse imaging techniques. For example, optimal imaging using PEG labeled with a near infrared dye (IRDye 800 CW) and MRI imaging using an HPMA copolymer labeled with an MRI contrast agent (DOTA/Gd^{3+}) (Wang *et al.* 2004) demonstrated that both polymer systems accumulated in the joints of arthritic mice.

Due to passive targeting, the application of PCs in drug delivery is expected to increase the drug concentration at the inflammation site while reducing the drug amount in healthy tissues. Thus, molecules with high anti-inflammatory potential but poor biodistribution may become promising drug candidates when conjugated to polymer backbones.

While the drug concentration in inflamed tissues can be increased through passive targeting, directing nanotherapeutics to a specific cell type by the incorporation of targeting ligands into its structure, i.e.

active targeting, may further improve therapeutic efficiency. Activated macrophages, which play an essential role in CID development and progress, over-express folate receptors and nanomedicines bearing folate have been shown to be actively internalized by these cells (Low and Antony 2004). Angiogenesis and enhanced cell-infiltration at the inflammation site is associated with the over-expression of adhesive molecules, such as integrins, on the surface of macrophages and vascular endothelial cells. The integrin-targeted nanomedicines utilizing cyclic forms of RGD oligopeptides exhibited enhanced anti-inflammatory properties *in vivo* (Koning *et al.* 2006).

Polymer conjugates with anti-inflammatory activity

The successful treatment of chronic inflammation poses considerable challenges to modern medicine, including gradual and controlled cellular death of specific immune cells without interfering with healthy tissues. The concept of drug conjugation can be widely applied within the context of inflammation, enabling drug accumulation at inflammation sites and controlled release in target tissue or cells. Therefore, several polymer conjugates with diverse anti-inflammatory drugs have been designed and their biological activity has been evaluated.

Some of the most frequently used and most potent anti-inflammatory drugs are glucocorticoids (GC), e.g. dexamethasone (Dex). However, the long-term application of free GC induces severe side effects. Therefore, a HPMA copolymer conjugate with Dex bound *via* a pH-sensitive bond was synthesized. The pH-sensitive hydrazone bond between the polymer backbone and Dex prevented drug release at pH 7.4 (corresponding to the blood pH) and enabled sustained Dex release in the acidic environment (pH 4.4-6) which is present at the site of RA; 14 % of the drug was released within 14 days at pH 5. Compared to free Dex, the Dex-polymer conjugate exhibited longer lasting and enhanced joint protection and anti-inflammatory effect in an *in vivo* rat model with arthritis, resulting in complete restoration of ankle size within 3 days (Liu *et al.* 2008).

The PEG backbone also served as polymer carrier for Dex bound *via* a pH-sensitive hydrazone bond. To overcome the scarcity of PEG conjugation sites, a linear multifunctional PEG-Dex conjugate was synthesized by the reaction of the functionalized short PEG chain with Dex-bearing monomers using copper-catalyzed click chemistry. Despite the same chemical structure of the biodegradable linker, the Dex release was much slower for PEG conjugates than for HPMA copolymer conjugates (the release rate from PEG-Dex conjugates was 0.5 % per day, while for HPMA copolymer-Dex conjugates it reached 1 % released Dex per day), probably due to the different chemical vicinity of the hydrazone bond. Therefore, the anti-inflammatory activity of the PEG-Dex conjugate was milder than in the case of HPMA copolymer-Dex conjugate, leading to only partial amelioration of joint inflammation in a rat model of arthritis (Liu *et al.* 2010).

Apart from acidic cleavable GC polymer conjugates, enzymatically cleavable linkers were utilized to conjugate diverse GCs to polymer backbones. Dex conjugate with poly(1-vinyl-2-pyrrolidone) with an esterase-sensitive spacer was synthesized by copolymerization of the Dex-bearing monomer and 1-vinyl-2-pyrrolidone (Timofeevski *et al.* 1996). Dex and other GCs were conjugated *via* ester linkers to diverse hydrophilic polymers, e.g. dextran (Penugonda *et al.* 2008), chitosan (Onishi *et al.* 2011), poly((dimethylamino)ethyl methacrylate) (Keely *et al.* 2009) or dendrimers (Inapagolla *et al.* 2010). However, their application *in vivo* and controlled release at the inflammation site is rather questionable given the abundance of esterase in the plasma.

NSAID, e.g. indomethacin, ibuprofen, naproxen, are widely used to reduce inflammation and pain in CID. However, their long-term systemic application may cause severe gastrointestinal difficulties. To enhance anti-inflammatory activity and reduce side effects, naproxen was conjugated to a PEG-based carrier *via* oxidative-sensitive spacer, a phenylboronic ester, cleavable after exposure to ROS, which are over-produced in inflamed tissue; up to 92 % of the drug was released in the presence of H_2O_2, while almost no drug release was observed in the absence of ROS (Qiu *et al.* 2015). A macromolecular prodrug of naproxen based on PAMAM dendrimers was designed to improve oral bioavailability. Naproxen-dendrimer conjugates with a lactate ester linker exhibited enhanced transepithelial transport, stability in human plasma and controlled release in liver cells (Najlah *et al.* 2007). Naproxen or other NSAIDs, e.g. indomethacin, have been conjugated to natural biodegradable polymer carriers, i.e. dextran (Mária *et al.* 1986) or chitosan (Isabella *et al.* 1996), *via* an ester linker cleavable by esterase-catalyzed hydrolysis. However, their wide application in inflammation treatment has not been realized.

Polymer conjugates with NSAIDs have gained interest in colon-specific drug delivery intended for IBD. To obtain a colon-specific delivery, azoreductase enzymes, selectively present in the colon, were exploited by introducing an azo linker between the polymer carrier and the drug molecule. An anti-inflammatory drug widely used in IBD treatment, 5-aminosalicylic acid (5-ASA), was conjugated to various water-soluble polymer carriers *via* an azo linker cleavable by colonic microflora (Philip and Philip 2010). The bioadhesive HPMA copolymer-5-ASA conjugate was prepared by copolymerization of an azo-linked 5-ASA-containing monomer with HPMA and the fucosylamine-containing comonomer (the bioadhesive component). The introduction of the fucosylamine moiety enabled enhanced adherence to guinea pig colon due to the site-specific binding of carbohydrate moieties complementary to colonic mucosal lectins. The incorporation of 5-ASA-bearing aromatic side-chains into HPMA copolymers enabling colon-specific drug release further increased their adherence probably through the combination of non-specific hydrophobic binding and specific recognition (Kopečková *et al.* 1994).

Analogously, 5-ASA conjugated *via* an azo linker to short PEG chains of M_w of 4,000 g/mol exhibited satisfactory drug release in the colon with minimized drug release and absorption in the upper intestine. The application of the PEG-based 5-ASA conjugate led to an enhanced anti-inflammatory effect, including bodyweight gain, improved tissue histology and proinflammatory cytokine decrease, *in vivo* using mice with induced colitis (Canevari *et al.* 2009). In addition, colon-targeted conjugates with 5-ASA or other NSAIDs based on PAMAM dendrimers (Wiwattanapatapee *et al.* 2003) or polysaccharides, including hydroxypropyl cellulose, dextran or chitosan, have been designed and synthesized (Larsen *et al.* 1989, Zou *et al.* 2005).

CID treatment often requires the application of immunomodulating agents, especially methotrexate (MTX), since the disease is often associated with autoimmunity. Most PCs with MTX were initially developed for cancer therapy. However, their application in anti-inflammatory CID treatment is very promising. Due to the accumulation of macromolecules in inflamed tissues, the long circulation time of albumin (19 days) and controlled MTX release by enzymes overexpressed in inflamed synovium (i.e. cathepsin B and plasmin), an MTX conjugate of human serum albumin ($M_w \approx 67,000$ g/mol) exhibited enhanced *in vivo* activity in murine collagen-induced arthritis (Fiehn *et al.* 2008).

Protease-cleavable oligopeptide linkers have been also utilized in the design of MTX conjugates of HPMA copolymers or hyaluronic acid. To enhance its anti-inflammatory potential, an HPMA copolymer-based MTX conjugate was decorated with biotin molecules for active targeting to macrophages. The oligopeptide spacer (GFLG) of the HPMA-copolymer conjugate enabled MTX release in lysosomes after the cellular uptake of the PC. However, its *in vivo* activity has not been demonstrated yet (Russell-Jones and Mcewan 2004). The hyaluronic acid conjugate of MTX utilized two lysosomal enzyme-cleavable linkers, i.e. GFLG and NFF. Despite MTX release from both conjugates by lysosomal enzymes *in vitro*, significant reduction in knee swelling of rats with arthritis was observed only after intra-articular injection of the conjugate with an NFF spacer (Shin *et al.* 2014).

Active targeting to folate receptors in macrophages was employed for drug delivery to the inflammation site using MTX conjugates of PAMAM dendrimers or biodegradable dextrans (Qi *et al.* 2015, Yang *et al.* 2016). The introduction of folic acid (FA) as a targeting moiety enhanced the cellular uptake of both conjugates into macrophages *in vitro*. The PAMAM conjugate of MTX showed a similar preventive effect on the development of arthritis in rats to free MTX; however, no spleen toxicity was observed for the MTX conjugate in comparison to the free drug. A biodegradable dextran-MTX conjugate with FA possessed improved biodistribution at the inflammation site and stronger remission of arthritis in mice through the inhibition of proinflammatory cytokines in comparison to both free MTX and the non-targeted corresponding MTX conjugate.

Polymer-promoted inflammation and immunogenicity

Immunogenicity is defined as the ability of a substance to provoke an immune response. An immune response could be desirable, in the case of vaccines, or could be an unwanted side effect of the material. When polymers are used as anti-inflammatory drug carriers, the activation of the immune system should be as low as possible (Farrera and Fadeel 2015).

Polymer physicochemical properties determine their interaction with the immune system. The majority of

nanosized drug delivery systems are based on non-water soluble materials, e.g. polyester or metal nanoparticles, carbon nanotubes, which are generally coated with hydrophilic polymers, i.e. PEG, HPMA copolymers, in order to improve the system biocompatibility, non-toxicity and non-immunogenicity (Dobrovolskaia and McNeil 2007). If they are not protected by coating, e.g. with PEG, they interact with plasma proteins, which makes them easily recognizable for immune cells, especially macrophages (Owens and Peppas 2006). Nevertheless, the majority of polymer-drug conjugates are based on hydrophilic polymers and therefore they are supposed not to induce undesired immune reactions *in vivo*. One of the most frequently used polymers in PCs has been developed as a safe plasma expander and in addition the biocompatibility and non-immunogenicity of HPMA copolymer-based carriers have been repeatedly shown *in vivo* and clinical trials (Gaspar and Duncan 2009, Říhová and Kovář 2010).

PEG-based therapeutics has received a great deal of interest in the delivery of therapeutic proteins in clinical practice (Jevsevar *et al.* 2010). PEGylation of these proteins considerably prolongs their blood circulation, thereby maintaining their biological activity. Despite its high popularity in nanotherapeutics design, several studies have shown that the repeated application of PEG may lead to immune response and formation of anti-PEG antibodies (Zhang *et al.* 2016).

The surface charge of PCs is one of the key factors influencing the immune response of the organism to PC application. Positively or negatively charged polymers are usually quickly entrapped by RES and eliminated by the liver (He *et al.* 2010).

The influence of the polymer carrier on the immune system is crucial in the design of anti-inflammatory drug-polymer conjugates, since unbalanced chronic inflammation is very sensitive to any external or internal proinflammatory stimuli. Therefore, we believe that PCs based on biocompatible non-immunogenic synthetic polymers, e.g. HPMA copolymers, would be very promising in the treatment of various CIDs.

Conclusions

Chronic inflammation is believed to cause numerous severe diseases, including RA, MS or some cancers, and its successful resolution often remains problematic. Polymer-drug conjugates based on hydrophilic and biocompatible materials have attracted attention in the modern treatment of inflammation or cancer due to targeted drug delivery and controlled drug release. We have reviewed the relationship between inflammation and inflammatory-related diseases with polymer conjugates, emphasizing the anti-inflammatory activity of polymer-drug conjugates and potential polymer-promoted inflammation and immunogenicity.

Conflict of Interest
There is no conflict of interest.

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