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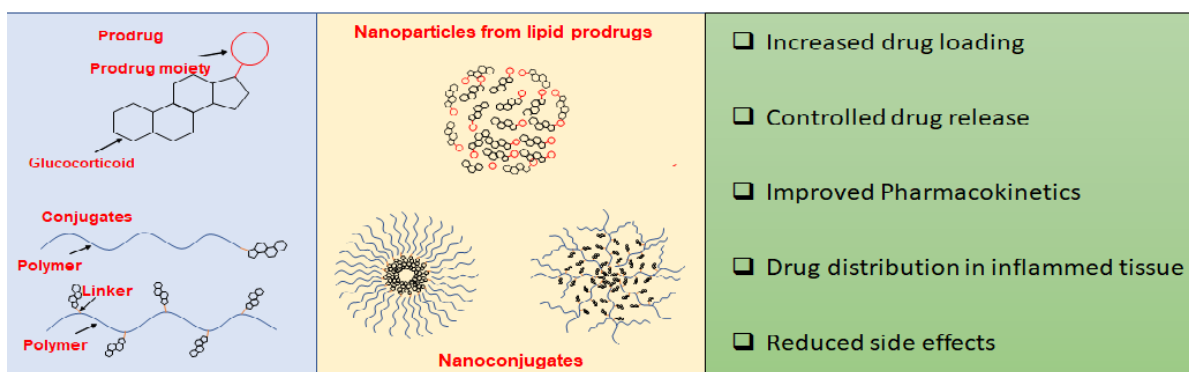
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Emerging nanoparticle platforms to improve the administration of glucocorticoids

Barbara Tessier, Nicolas Tsapis, Elias Fattal*, Laurence Moine*
Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 91400, Orsay, France.

*Both senior and corresponding authors
Université Paris-Saclay
Institut Galien Paris-Saclay
UMR CNRS 8612
Henri Moissan, 670
17, Avenue des Sciences
91400 Orsay
France



Abstract

Glucocorticoids (GC) are common drugs used to treat acute and chronic inflammatory diseases, whose prolonged use can result in severe side effects hampering their efficacy. In addition, the pharmacokinetics, and biodistribution of GC are inadequate to support high efficacy with reduced toxicity. Following the marketing of GC prodrugs, new GC prodrug entities, and conjugates, have been developed. These new prodrugs and conjugates have been administered in free form or under a nanoparticulate form for local or systemic administration. These **nanoparticles from lipid prodrugs** and nanoconjugates change the paradigm of GC delivery, solving the issue of low drug loading into nanoparticles and circumventing the **potential** burst release effect by allowing a more controlled delivery of the GC and better targeting in inflammatory sites. This review highlights the design strategies, recent advances in GC prodrugs and conjugates, and their delivery in nanoparticulate form, demonstrating the strong potentialities of these novel strategies.

Keywords

Glucocorticoids; prodrugs, nanoparticles from lipid prodrug, conjugates, nanoconjugates, inflammation

Highlights

- **Nanoparticles from lipid prodrugs** and nanoconjugates allow high drug loading and controlled release of glucocorticoids
- **Nanoparticles from lipid prodrugs** and nanoconjugates **better** target inflammation sites in **of several** inflammatory diseases
- Inflammation-responsive delivery systems hold great promise for spatio-temporal control of glucocorticoid delivery

1 Introduction

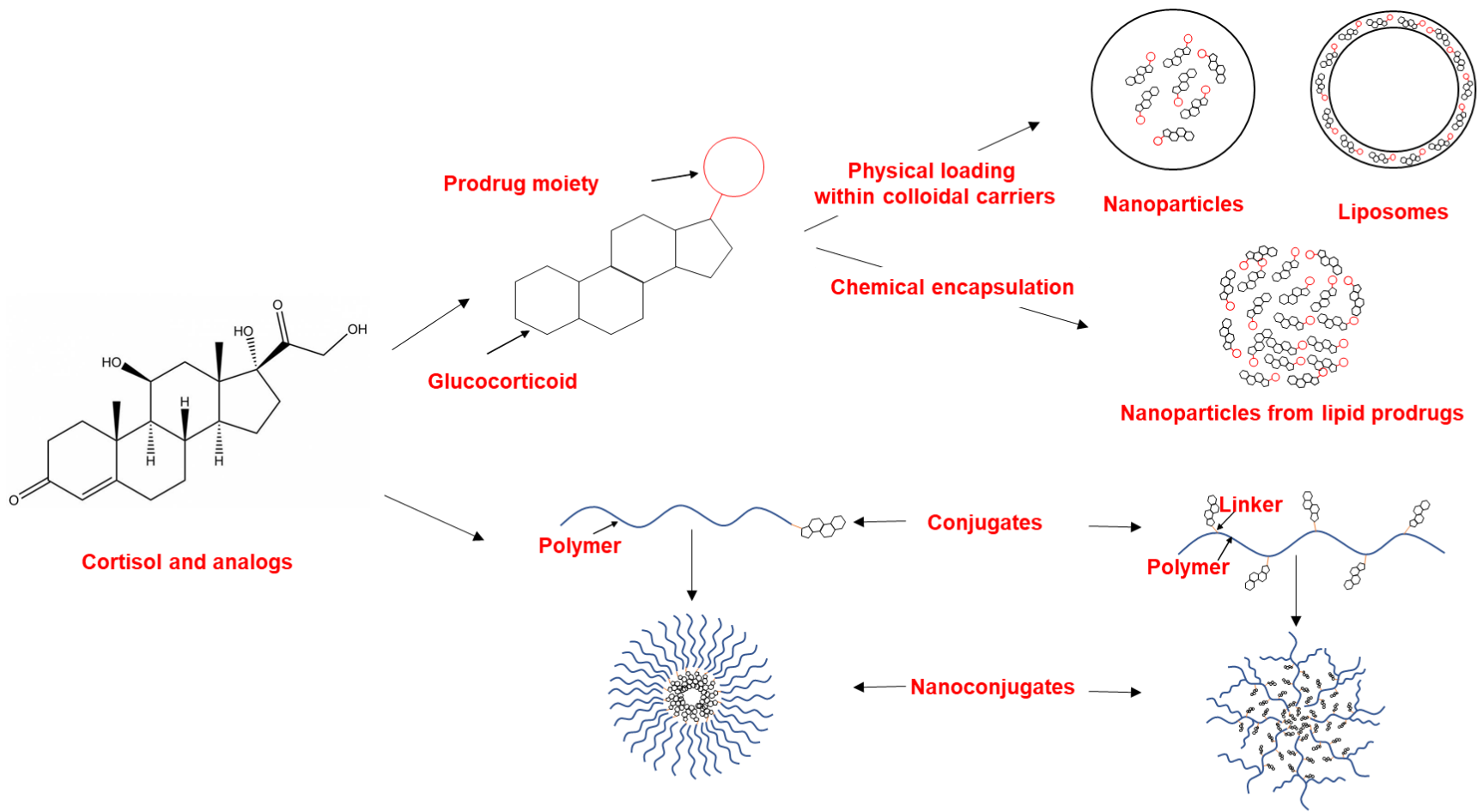
Glucocorticoids (GC) are highly efficient drugs widely used to treat inflammatory and autoimmune diseases. Routes of administration are oral, parenteral, or topical. However, their use is often associated with severe side effects such as hyperglycemia, edema formation, gastrointestinal disturbances, osteoporosis, and amyotrophy [1]. They can also induce inhibition of the hypothalamic-hypophyseal axis in case of prolonged treatment and thus engage the patient's vital prognosis by causing an acute adrenal insufficiency [2]. Therefore, their use in treating chronic diseases is closely monitored and limited by the inevitable occurrence of these adverse effects.

Most GC have good oral bioavailability and are eliminated mainly by hepatic metabolism and renal excretion of the metabolites [3]. However, most of the concerns in the delivery of GC are related to their pharmacokinetics and biodistribution properties that are inadequate to achieve high efficacy at a low dose [4]. Indeed, for most administration routes, GC are quickly cleared and taken up mainly by the liver, where they are metabolized [5,6].

Both chemical modifications and drug delivery approaches have been developed to reduce the volume of distribution and the off-target accumulation of GC, thereby lowering their toxicity and increasing and prolonging drug levels at the pathological site to improve their therapeutic efficacy. Indeed, novel approaches to develop prodrugs or conjugates are now being considered. Prodrugs consist of attaching a **pharmacologically inert** moiety to the drug itself. The whole **new molecule** is then metabolized in the body to produce the pharmacologically active drug (Fig. 1). In the specific case of GC, the moiety consists of phosphate or acetate groups or short and long fatty acids. In contrast, conjugates involve the attachment of GC drugs as hydrolytically releasable pendant groups along a polymer backbone or as a single GC drug molecule at the end of the polymer chain (usually PEG chain) (Fig. 1).

In many cases, prodrugs or conjugates, due to their amphiphilic nature, can self-assemble. They produce nano-assemblies in the form of micelles, vesicles, or nanoparticles that are denominated **"nanoparticles from lipid prodrugs" (NLP)** or **"nanoconjugates"** (Fig. 1). Both possess some exciting common advantages. One can tailor a GC prodrug or conjugate **to promote its insertion** into a polymer or lipid carrier **to a higher extent than** free GC. Moreover, many prodrugs or conjugates can self-assemble into nanoparticles solving the issues of poor encapsulation and burst release in biological fluids. In addition, nanoparticles can display a long-circulation time in the blood compartment, which modifies their tissue distribution, favoring extravasation at inflammatory sites. This review highlights the design strategies,

recent advances in glucocorticoid prodrugs and conjugates, and their nanoparticle delivery demonstrating these novel strategies' strong potential.



From cortisol to nanoparticles from lipid prodrugs and nanoconjugates

Fig. 1. Design strategies for glucocorticoids delivery in nanoparticulate form

2 Rationale for the design of novel delivery strategies for Glucocorticoids

GC constitute a homogeneous family of therapeutic molecules derived from cortisol, an endogenous hormone circulating in humans. Their anti-inflammatory and immunosuppressive action gives them a high therapeutic potential for treating many acute or chronic inflammatory and autoimmune diseases. They can also act as adjuvants to chemotherapies to reduce their adverse effects [7]. Classical genomic actions are the essential mechanisms of GC action leading to modifications in gene expression. These actions are due to the passage of GC molecules across the plasma membrane, their high affinity binding to inactive cytosolic GC receptors (cGCR), the formation of the activated GC/cGCR complex, and translocation of the complex to the nucleus where it regulates gene expression [8]. GC can modulate the transcription of various genes, including cytokines and chemokines, receptors, enzymes, adhesion molecules, and inhibitory proteins [9]. These steps are necessary for producing most cellular responses to glucocorticoids [9]. Because of the rapidity of some GC effects, the concept that non-genomic actions may contribute to GC mechanisms of action has emerged. Many of these mechanisms have been described elsewhere and reviewed [10].

GC are predominantly hydrophobic molecules that are poorly soluble in biological fluids. However, this drawback was partly overcome by attaching an acetate or phosphate group, as defined in section 3. This poor solubility induces a very short plasma half-life and a high liver uptake [5]. Another disadvantage of free GC is their largely uniform and unspecific distribution within the human body, which can result in side effects and limited drug availability in target tissues [5]. The prolonged use of GC is associated with severe side effects, including osteoporosis, glucose intolerance, serious infections, diabetes, hypertriglyceridemia, gastritis, peptic ulcer disease, GI bleeding, skin thinning, purpura, cataracts, glaucoma, accelerated atherosclerosis, hypertension and cardiovascular diseases [11].

The research and advances have evolved in different steps, as presented in Fig. 1. It is today believed that i) modifying pharmacokinetics properties of GC by increasing their plasmatic half-life, ii) reducing the frequency of administration as well as the given dose, and iii) changing the tissue distribution will strongly impact their efficacy and reduce the side effects. The concepts of conjugates, prodrugs, and their nanoparticle counterparts are described in Fig. 1. These delivery systems use the pathophysiological aspect of inflammation for targeting and/or controlled release. Regarding tissue targeting, an enhanced permeability and retention (EPR) effect was identified in different inflammatory diseases [12]. Indeed, during inflammation, an increased vascular permeability coupled to a

deregulated neovascularization occurs. This EPR effect was first discovered in tumors [13] but occurs similarly in inflammatory diseases leading to extravasation of macromolecules or nanoparticles with however one essential difference, which consists in a retention mechanism that happens due to inflammatory cell sequestration as opposed to retarded lymphatic drainage in solid tumors. For this reason, this effect is known as extravasation through leaky vasculature and the subsequent inflammatory cell-mediated sequestration (ELVIS) [14].

Other parameters of pathologically inflamed tissue can be considered when designing inflammation-responsive drug delivery systems. In inflammatory sites, the pH is significantly reduced, from 7.4 in healthy tissue, to around 6.4 [15]. This reduction is due either to the bacterial metabolites in the case of infection-associated inflammatory processes or to the hypoxic conditions that induce a shift toward anaerobic glycolysis and lactate formation [16]. Added to pH changes, an enhanced ROS generation by polymorphonuclear neutrophils occurs at inflammatory sites, with level increasing from 10- to 1000-fold more than in normal human plasma [17]. An overexpression of inflammatory and matrix-metalloproteinases (MMPs) (particularly MMP-2 and MMP-9) [18], ureases, cathepsins, elastases [19], or phospholipases A2 (PLA2s) [20] takes place. Finally, in some cases, heat, one important symptom in inflammation, occurs particularly at the skin surface [21,22].

3 Glucocorticoids prodrugs and nanoparticles from lipid prodrug

3.1 Marketed prodrugs

The basic configuration of glucocorticoid consists of four-fused rings associated with one cyclohexadienone ring, two cyclohexane rings, and one cyclopentane ring. Numerous short- and long-acting molecules are produced via pharmacomodulation performed on the cortisol pharmacophore. Indeed, there is a strong relationship between the structure of the molecules and their pharmacological activity. These modifications aim to increase the anti-inflammatory glucocorticoid action of the molecules, which is favorable to the therapeutic effect, and to decrease the mineralocorticoid part, due to the retention of sodium ions, which is responsible for many undesirable effects [23]. For instance, the 9- α position hydrogen can be replaced by a fluorine such as in dexamethasone and betamethasone. Prednisolone results from adding a double bond between the C-1 and C-2 positions of cortisol (Fig. 2) [23].

Prodrugs were first designed to improve the aqueous solubility of GCs by grafting polar moieties such as sodium phosphate or hydrogen succinate (Fig. 2) [24]. Both esters are expected to be hydrolyzed *in vivo* to yield the active GC. They can facilitate oral absorption or produce liquid formulations such as oral solutions, eye drops, or injectable solutions. Oral solutions containing prednisolone sodium phosphate have been developed to improve

patient compliance, notably with children for which the prodrug has better palatability than the prednisolone base [25]. Oral solutions also ensure easy self-administration in emergencies like anaphylaxis or urticaria with angioedema [26].

There are a variety of uses for injectable GC prodrugs. For example, betamethasone acetate relieves osteoarthritis-induced inflammation after intra-articular injections [27]. At the same time, methylprednisolone succinate can be used in parenteral, intramuscular, or intravenous injections for adrenal insufficiency, anaphylactic shock, or angioedema, among other indications.

Following parenteral administration, the onset of action of GC is strongly linked to the pharmacomodulation of the molecule. Thus, water-soluble derivatives such as phosphates or hemisuccinate have an immediate action start. On the contrary, esters or acetonides have a delayed effect with a long duration of action that can reach up to 3 weeks. GC prodrugs have been achieved by adding long carbon side chains such as dipropionate, acetonide, or valerate moieties to GC structure to allow cutaneous, inhaled, or nasal administration. This simple modification has led to many commercial products. Beclomethasone dipropionate and mometasone furoate are used for nasal or inhaled administration to treat allergic rhinitis or asthma [28,29]. Alclometasone dipropionate and fluocinolone acetonide are used for dermal administration to reduce swelling, redness, and itchiness in skin diseases such as eczema and dermatitis [30]. In the case of topical corticosteroids, with dermal, nasal, or inhaled administration, their increased lipophilicity ensures higher local retention by the nasal mucosa, lung tissue, or skin due to a slower dissolution, thereby reducing systemic exposure and potential side effects [31,32].

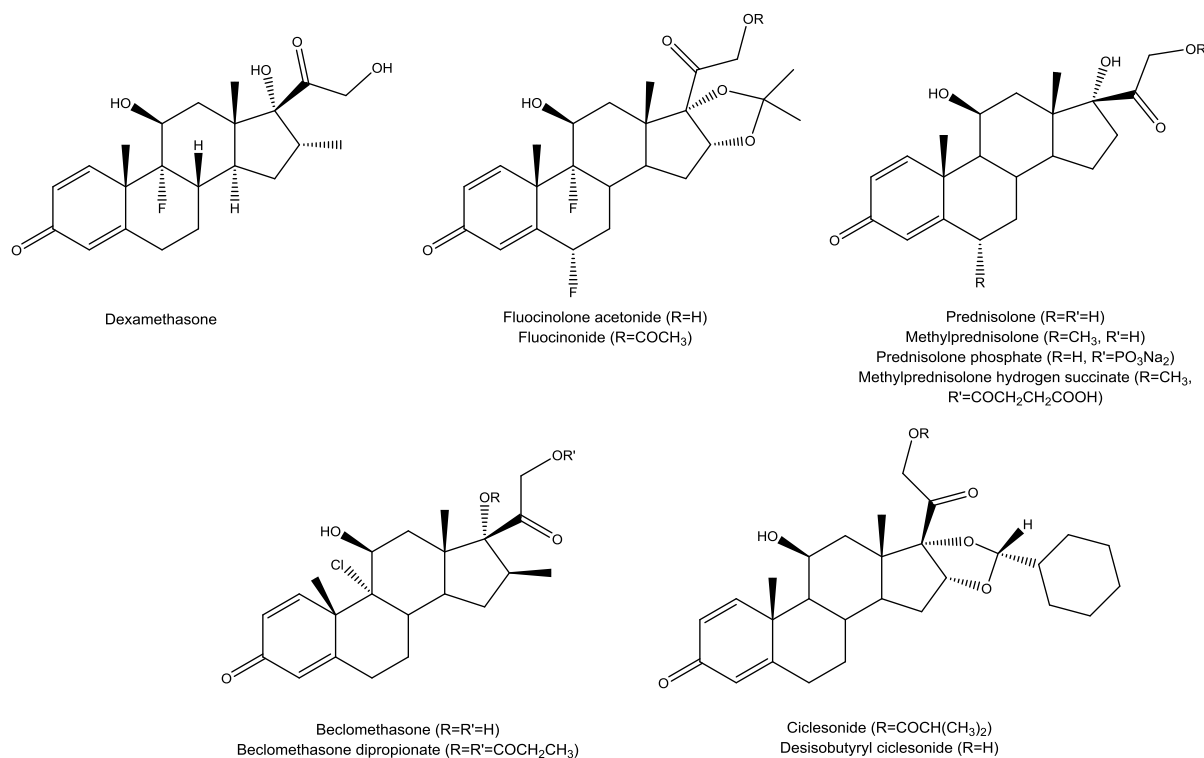


Fig. 2. Examples of glucocorticoid and glucocorticoid prodrugs.

Despite all the advantages provided by prodrugs to improve the therapeutic index of GC, the prodrug moieties may induce toxic side effects. For instance, phosphate groups can produce hypocalcemia at high concentrations, causing mild pruritus and paresthesia at the administration site [33]. Moreover, neutral prodrugs of ionically charged drugs might lead to intracellular entrapment and, thus, toxicity following their release [34], as charged compounds have difficulty crossing the cell membrane. This effect might be undesirable in inflammatory diseases and limit the extensive use of these marketed prodrugs.

3.2 Nanoparticulate delivery of GC prodrugs

As seen above, lipophilic prodrugs improved the efficacy of GC given by local administration to treat inflammation in specific sites. Although some prodrugs that are given systemically have a delayed effect, they are still unsuitable for diseases with multiple inflammatory lesions such as **rheumatoid arthritis** or multiple sclerosis. It is not feasible to locally inject the prodrug at each inflammation site [35,36]. Besides, like most low molar mass prodrugs with small moieties, GC prodrugs have a non-specific distribution in the body, accumulating in healthy tissues instead of reaching inflammation sites. Thus, these prodrugs suffer from the same shortcomings as any free compound, resulting in the appearance of side effects and less anti-inflammatory activity in the target tissues [37].

Improving GC targeting **can be obtained by using** nanoparticles [38]. They physically encapsulate the drug in the particle core or shell and provide a sustained drug release,

increased circulation half-life, better distribution at the inflamed site, and reduced undesirable side effects [39]. They are prepared with natural or synthetic polymers, lipids, or inorganic materials able to extravasate through the ELVIS effect [14,40].

3.2.1 Liposomes and lipid nanoparticles

Initially, the most common drug-loading strategy was to incorporate GC into the lipid bilayer of liposomes due to their lipophilicity. However, GC were not sufficiently lipophilic to avoid leakage of the drug. This resulted in an unstable encapsulation, as shown by Mishina *et al.* [41], where 70% of GC had leaked after one week. New strategies employing GC prodrugs were developed to resolve this issue. One of the first approaches to creating a GC prodrug-liposome is to formulate lipid vesicles with an aqueous core containing a water-soluble prodrug. Active loading of methylprednisolone hemisuccinate and betamethasone hemisuccinate was achieved [42–44]. These liposomes have been developed mainly for intravenous administration to treat rheumatoid arthritis or autoimmune diseases like multiple sclerosis. In models of arthritis, liposomal formulations effectively reduced inflammation with only a single administration instead of a daily administration of free GC over a week, ensuring a continued anti-inflammatory effect [45]. Other observed benefits included reduced bone degeneration, absence of hyperglycemia, and potentially shorter adrenal suppression [45]. It was shown that the higher therapeutic activity of encapsulated GC prodrugs over the free compound could be attributed to a preferential accumulation in inflamed joints [46].

Similar results were obtained with models of multiple sclerosis in which liposomes encapsulating prednisolone phosphate were superior to a 5-fold higher dose of free GC. The administration of the liposome formulation resulted in higher and persistent drug levels in the spinal cord through passive targeting, with no adverse side effects [47]. Due to sustained therapeutic efficacy, a single dose of liposomes was also more efficient than free methylprednisolone pulse therapy. For this reason, the treatment was considered more convenient for chronic inflammatory diseases [48]. A novel system based on liposome-encapsulating layered double hydroxide nanosheets were developed by Zhang *et al.* [49]. Diffusion through the nanohybrid and liposome layers achieved a sustained betamethasone dipropionate release. While these liposomes improved the delivery of GC prodrugs, they have a poor loading efficiency (< 2 wt%), probably due to the passive nature of the encapsulation process and the small volume of the aqueous core. An alternative approach has been proposed to increase the loaded amount through an active process using a gradient. Avnir *et al.* [42] managed to boost the methylprednisolone hemisuccinate (MPS) loading in PEGylated liposomes by remote-loading the drug through a transmembrane calcium acetate gradient. Protonated MPS diffused through the lipid bilayer, after which it

was trapped as an MPS-calcium salt precipitate in the inner aqueous compartment of the liposome. MPS precipitation stabilized its loading into the liposome, prevented leakage, and ensured the slow release of the drug after administration [46,47]. *In vivo* studies showed that these MPS liposomes had a much longer half-life than the free drug. They induced a substantial accumulation in inflamed paws, provided a slow release of the drug, and efficiently induced remission of arthritis with a 5-fold lower dose than free MPS with no evidence of side effects [46,47].

Hydrophobic prodrugs are primarily used for local treatments of diseases such as atopic dermatitis and inflammatory lung disease. Liposomes encapsulating betamethasone dipropionate [50], betamethasone valerate, or diflucortolone valerate were prepared for topical skin administration [51]. They successfully permeated through the stratum corneum of *ex vivo* samples. They remained in the epidermis and dermis layer with a ~3-fold higher retention than commercial creams while avoiding permeation through the following layers, thus potentially preventing systemic circulation and potential side effects. The liposomes had a sustained anti-inflammatory *in vivo* activity, higher than the commercial drugs even though they were applied with a 10-fold lower dose and presented no irritant effect, making them promising treatments for atopic dermatitis [51]. Nanostructured lipid carriers (NLC) consist of aqueous colloidal dispersion composed of blended solid lipids and liquid lipids (oils). Similarly to liposomes, betamethasone dipropionate-loaded NLC induced high drug skin retention *in vitro* and *in vivo*, low muscle tissue, and blood penetration [52]. In addition, they decreased skin irritation compared to the free drug [52].

Many liposomal formulations of beclomethasone dipropionate have also been designed for inhalation to treat inflammatory lung diseases [53–57]. Darwis and Kellaway showed that the drug encapsulation efficiency could be modulated by the type of phospholipids used to formulate the liposomes. Indeed, dilauroylphosphatidylcholine (DLPC) entering liposome composition induced a higher drug loading efficiency than other phospholipids with higher melting temperatures (T_m) and longer acyl chains. These latter are more rigid and therefore cannot accommodate the prodrug in the lipid bilayer. However, the loading rates were still relatively low, the highest being 3.7 wt% [53]. Similar nebulized DLPC and DPPC (dipalmitoylphosphatidylcholine) liposomes tested in healthy volunteers have shown that 80% of the initial dose of the drug remained in the lungs after 24 hours. They could potentially decrease the frequency of GC administration and thus reduce the risk of side effects [54]. Finally, micelles composed of distearoyl phosphatidylethanolamine coupled with polyethylene glycol (DSPE-PEG) encapsulating beclomethasone dipropionate were prepared to improve drug retention in the lung and to allow aerosol delivery through nebulization [58].

Instead of encapsulating existing GC prodrugs, other non-commercialized GC prodrugs have been developed specifically to formulate nanocarriers. They have been synthesized by the covalent bonding of a fatty acid to a GC and were further incorporated in liposomes [59–63]. It was shown that with a fatty acid chain corresponding to the phospholipids used to formulate liposomes (e.g., cortisol palmitate encapsulated in DPPC liposomes), drug retention in the liposome was higher, and release was longer [62]. The 21-palmitate derivatives of cortisol, prednisolone, and dexamethasone effectively reduce inflammation with a substantial accumulation in inflamed tissues and a significantly higher efficiency than the free-drug counterparts [59–61,63]. Liposomes have been revisited more recently to avoid burst release by increasing the affinity of the GC prodrug to the lipid bilayer. In these more recent formulations, DSPE-PEG has been added to protect the liposomes from plasma elimination and cholesterol to further stabilize the retention of the fatty acid GC in the lipid bilayer through condensation with a saturated fatty acid chain containing phospholipids [64]. *In vivo* experiments showed that these liposomes maintained a high concentration of prednisolone palmitate in circulation **as well as a high concentration of** the active drug after fatty acid cleavage by blood esterases [65]. A lipid micellar system was designed for the delivery of dexamethasone palmitate. Pharmacokinetic in rats and *in vivo* fluorescence imaging showed that mixed micelles had higher bioavailability and targeting efficiency in inflammatory sites improving the treatment of rheumatoid arthritis [66].

3.2.2 Polymer nanoparticles and micelles

Polymer nanoparticles have also been used to encapsulate water-soluble GC prodrugs such as betamethasone phosphate for IV administration to treat rheumatoid arthritis, chronic inflammation in asthma, and autoimmune uveoretinitis [67–69]. They provided the same benefits as liposomes, with an accumulation in inflammatory sites and sustained therapeutic efficacy. Dexamethasone palmitate was also entrapped in poly(lactide-co-glycolide) (PLGA) nanoparticles covered by PEG [70]. The pharmacokinetics profile of dexamethasone was improved after intravenous injection in a collagen-induced arthritis model in mice. Histological analysis revealed an improvement in the knee structure and reduced cell infiltration in animals treated with encapsulated dexamethasone palmitate compared with the soluble dexamethasone phosphate [70]. Polymeric nanoparticles have also been developed to improve the retention of GC prodrugs in the skin. PLGA or chitosan nanoparticles encapsulating betamethasone valerate showed improved retention of the drug in the epidermis [71,72]. Compared to a commercial cream with a 10-fold higher drug dose, a 2-fold higher amount of the drug was found in rat skin treated with lecithin/chitosan nanoparticles resulting in a higher anti-inflammatory action [71]. Increasing the drug

retention in the epidermis creates a drug reservoir, thus prolonging the residence time of the drug in the skin and ensuring a sustained therapeutic effect.

Physical encapsulation of GC prodrugs into lipid or polymer nanoparticles resulted in a significant improvement of biopharmaceutical properties of GC compared to the free prodrugs improving drug efficiency in preclinical models, particularly after intravenous administration. Moreover, nanoparticulate delivery of the prodrugs induced a reduction of side effects. Nevertheless, in many cases, the loading efficiency was very low [49,53,70], and the release was poorly controlled. However, a slight improvement of both properties was demonstrated when fatty acid-derived GC prodrugs were encapsulated [59–61,63–66].

3.3 Nanoparticles from lipid prodrugs (NLPs)

NLPs use chemical encapsulation instead of the physical encapsulation described above. It produces nanoparticles out of a prodrug solving two problems: the **poor drug loading** and the drug burst release. In this context, **our group has described a new process for obtaining GC nanoparticles from a prodrug made of a fatty acid derivative (palmitate) of dexamethasone or budesonide added to DSPE-PEG and obtained by the emulsion solvent evaporation method** (Fig. 3). The presence of DSPE-PEG [5,73] or other amphiphilic molecules such as poloxamer 407 [74] improve considerably the stability by reducing the Ostwald ripening effect involved in nanoparticle destabilization. Nanoparticles composed of dexamethasone palmitate and DSPE-PEG display a drug loading equivalent to free dexamethasone of 49 wt% [5,73]. *In vivo* experiments were carried out in a model of rheumatoid arthritis. There was a significant reduction in arthritis score with these nanoparticles compared to the free drug at a **dose of 1 mg/kg** [73]. A similar anti-inflammatory effect between a dose of 0.1 mg/kg administered with nanoparticles and 1 mg/kg of free drug was observed, showing that drug dosage can be reduced through encapsulation in a nanocarrier [73]. An exciting approach involved designing the same **NLPs** with a cationic lipid, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), covering their surface [75]. These nanoparticles allowed the combination of dexamethasone palmitate and an anti-TNF α siRNA to produce a synergistic anti-inflammatory effect more substantial than the one **obtained with each drug separately** [75]. In another **study**, budesonide palmitate was turned into nanoparticles using the same DSPE-PEG and DSPE-PEG coupled to mannose to target the mannose receptor on macrophages [76].

Although newly applied to the delivery of glucocorticoids, this approach has many advantages. It mainly allows the encapsulation into one particle of all the active drugs [5]. The hydrolysis could be controlled based on the type of prodrug moiety and chemical bond. However, despite the absence of a burst release effect, drug release might occur too quickly

before the nanoparticle reaches its target. This is the reason why it is necessary to determine the optimal length of the fatty acid chain and the type of **chemical bond** that controls the release kinetics. Moreover, since the **NLPs** are composed of the prodrug and a lipid coupled to polyethylene glycol (PEG), further studies should explore the immune response to PEG [77] that might modify the pharmacokinetics properties of the **nanoparticles** through the accelerated blood clearance (ABC) phenomenon similarly **as observed for liposomes** [78].

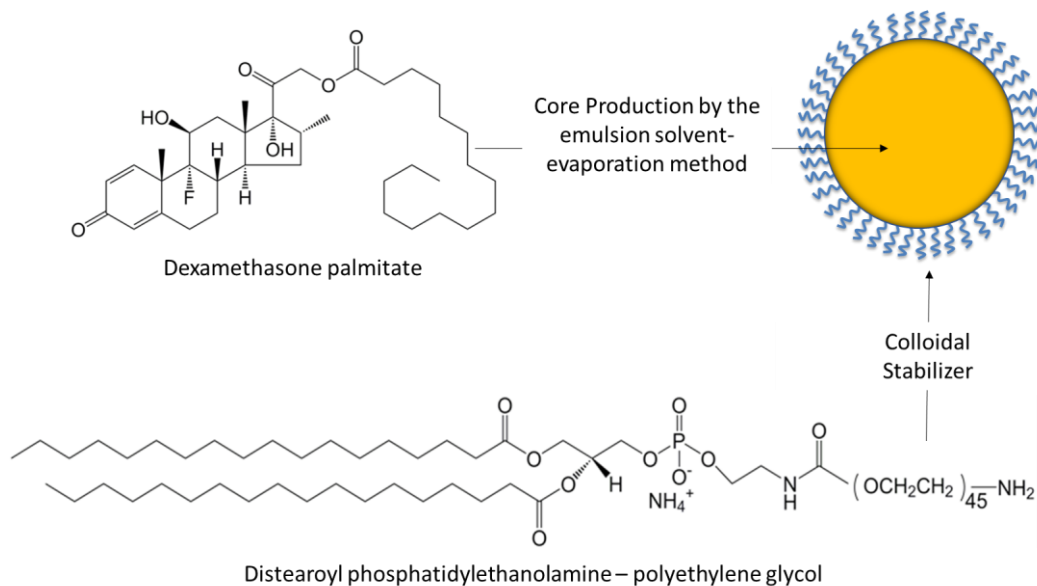


Fig. 3. Schematic description of nanoparticles obtained from lipid prodrugs.

4 Polymer-drug conjugates

The concepts of polymer-drug conjugates developed by Ringsdorf in the mid-1970s have paved the way for compounds with improved pharmacological properties and easy administration [79]. **These concepts involve either end-modification of a polymer with one drug molecule or the attachment of several drug molecules in the form of releasable pendant groups along the polymer backbone** (Fig. 4). These conjugates can be defined as “macromolecular prodrugs” that differ from regular prodrugs by the added moiety length on the drug and often by the number of drug molecules linked to the polymer carrier (Fig. 4). Due to the significant difference in size between a polymer chain and a small prodrug moiety, “conjugates” should be distinguished from smaller prodrugs. Indeed, although very different from “classical” nanoparticles, polymer-drug conjugates are considered in the nanometer size range.

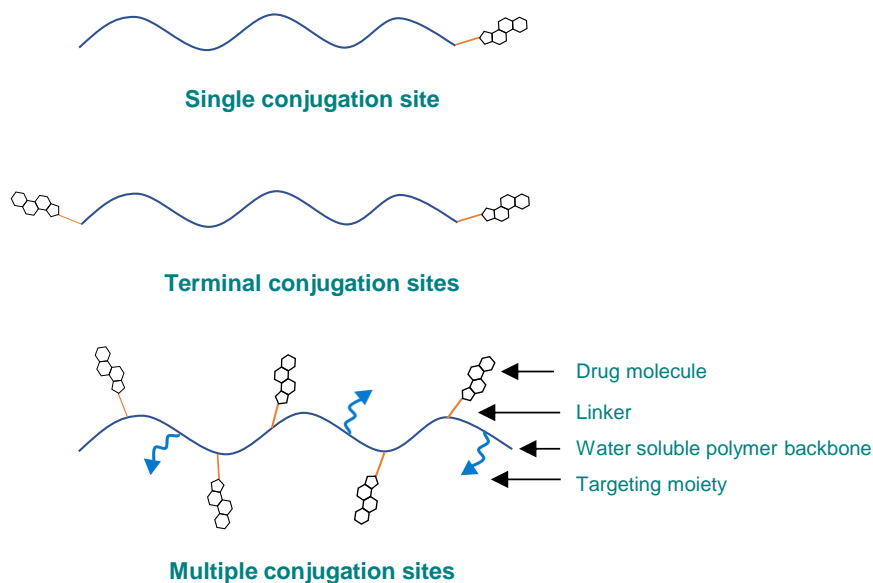


Fig. 4. Representation of polymer conjugates structures with varying quantities of conjugation sites.

Most recently developed polymer-drug conjugates focused on cancer therapy, although their use extends to many other diseases [80,81]. Like nanocarriers such as liposomes or polymer nanoparticles, conjugates can improve the solubility of hydrophobic compounds such as GC. They also allow passive targeting of the disease site while protecting the drug from degradation or early release, thus reducing toxicity to healthy tissues. Some polymers can also achieve active targeting by binding a targeting ligand. In addition, as opposed to conventional nanocarriers, which physically encapsulate drugs by solubilization or entrapment, and similarly to NLPs, conjugates offer a "chemical encapsulation" by covalently linking a drug to a polymer creating a "new chemical entity," as described by Duncan [79]. This covalent linkage reduces the burst release of the conjugated drug and ensures a longer sustained release through the cleavage of a covalent bond [82]. They also benefit from a prolonged retention time in circulation due to their higher molar mass, allowing passive targeting of inflamed sites through the ELVIS effect [83].

A linker, sensitive to pH, enzyme, ultrasound, light, or temperature, is sometimes introduced between the polymer and the drug to add an extra chemical reactive function to the conjugate, essentially modulating the release conditions of the drug [84]. Using stimuli-sensitive linkers presents the advantage of targeting specific intracellular compartments or tissue environments where an acidic pH or the presence of enzymes to metabolize the bond will ensure the localized delivery of the drug in those areas, thus reducing the risk of side effects [85–87].

Polymer-drug conjugates can be classified as either hydrophilic or amphiphilic conjugates. While hydrophilic conjugates can be administered as such, amphiphilic conjugates are not

soluble in water but can self-assemble into colloidal nanocarriers, forming "nanoconjugates" (Fig. 5).

The main advantage of the conjugate or nanoconjugate is its versatility and the myriad of possibilities with the various polymers and linkers that can be used. In addition, it can become a trustworthy multi-modal delivery platform by conjugating different types of compounds on the same polymer chain [88,89]. The possibility of linking more than one type of compound to the polymers expands the use of these nanocarriers to theranostic applications with a probe associated with active targeting through ligands. It also allows the co-delivery of various drugs on a single carrier [83,90].

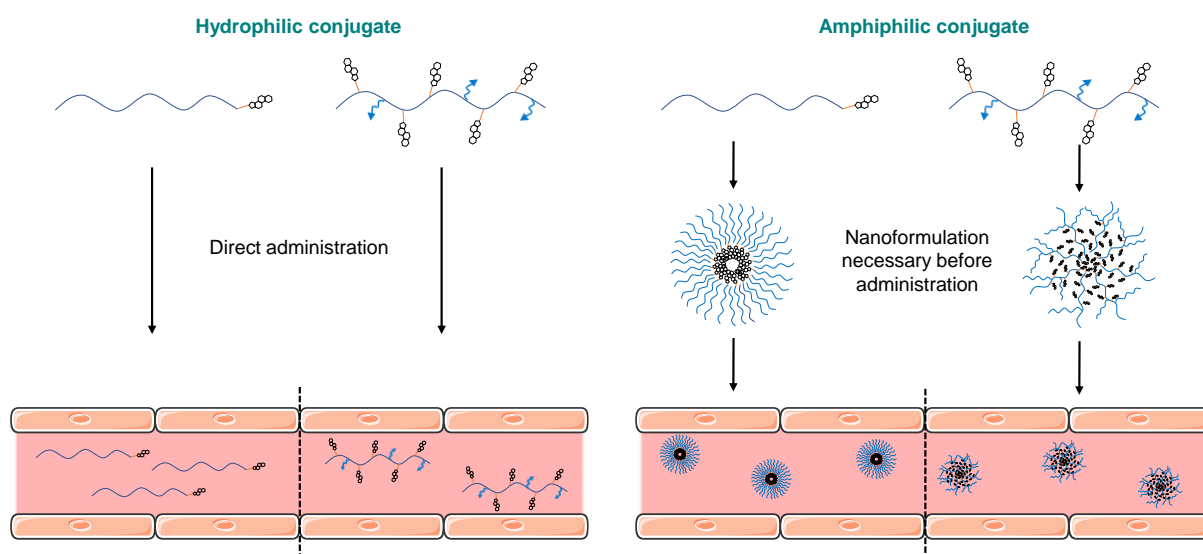


Fig. 5. Design of hydrophilic conjugates or amphiphilic nanoconjugates.

4.1 Hydrophilic conjugates

The most common conjugates developed for GC are hydrophilic as they are water-soluble and can be easily administered without further formulation. Examples of aqueous-soluble polymers used for GC drug delivery are presented in Fig. 6. Besides PEG and its derivatives, most polymers comprise repetitive units with reactive functions. A drug molecule or a linker can be conjugated to these functions, allowing multiple-molecule delivery. There are a couple of strategies to design polymer conjugates with various compounds. Often the drug is conjugated to the polymer after its synthesis, but the compound can also be linked to the monomers before polymerization to better control drug loading [91,92]. As mentioned earlier, a linker is often inserted between the drug and the polymer chain to control drug release. Two main groups of conjugates have been developed to deliver GC: pH-sensitive conjugates and enzymatic-cleavable conjugates.

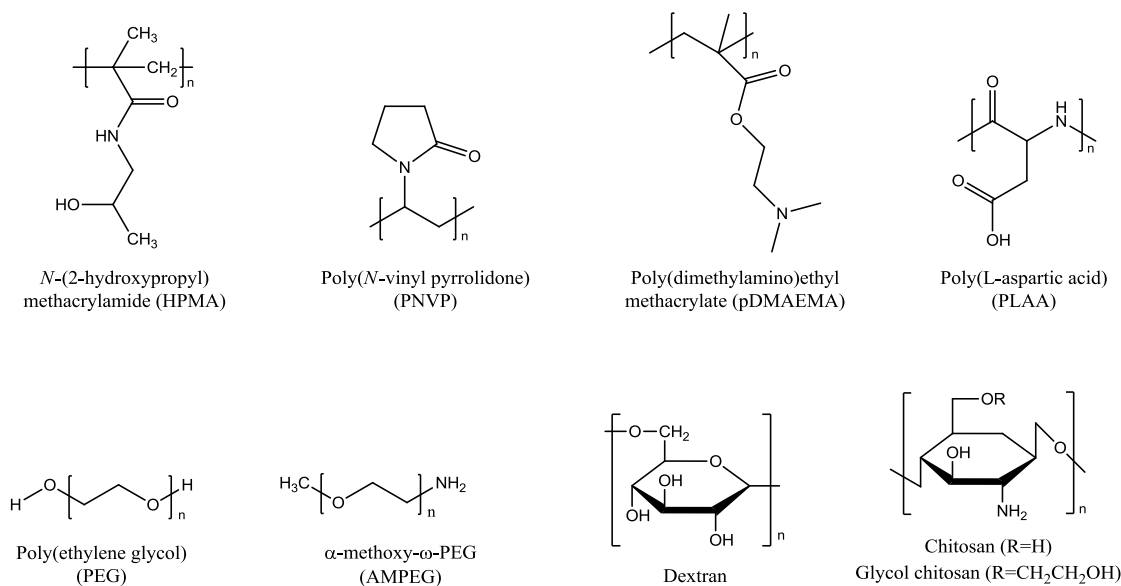


Fig. 6. Aqueous-soluble polymers used to form hydrophilic conjugates with glucocorticoids.

4.1.1 pH-sensitive conjugates

Most pH-sensitive conjugates carrying an acid-cleavable linker would be appropriate for localized release of GC (Table 2) to treat inflammatory diseases such as rheumatoid arthritis. Indeed, sites of inflammation, including inflamed joints, present an acidic environment due to increased metabolic activity and lactate production [93]. Furthermore, the acidic lysosomal content of macrophages or synoviocytes could also trigger drug release after cellular uptake of the conjugates. By linking the drug to the carrier through an acid-cleavable linker, localized release in inflamed areas with acidic pH or a higher number of pro-inflammatory cells, such as macrophages, could reduce the offsite activity of the drugs.

N-(2-hydroxypropyl)methacrylamide (HPMA) is one of the main polymers developed as a conjugate to treat inflammatory disease. It has been extensively studied as a drug carrier as it is a biocompatible, non-toxic, and non-immunogenic polymer [94–96]. It has been used to improve dexamethasone delivery after IV administration. A pH-sensitive monomer of dexamethasone bound to N-methacryloyl glycyglycine hydrazide was synthesized before copolymerization with HPMA [97,98]. This strategy reduced batch-to-batch variations in drug loading, as previously observed when dexamethasone grafting was performed after HPMA polymerization and not simultaneously with synthesis [99]. The formation of a hydrazone bond between dexamethasone and HPMA polymer maintains the conjugate stable at neutral pH and avoids the premature plasmatic release of the drug [100]. Due to its high molecular weight and stability at neutral pH, preferential accumulation was observed in inflamed joints compared to normal joints due to a longer **blood** circulation time of the conjugates compared to the free drug. A single administration of the conjugate with a dexamethasone content of 9

wt% was as effective as a daily administration of the free drug for 30 days to treat inflammation. Interestingly, while inflammation returned after the cessation of free drug injections, it remained suppressed by the conjugate, showing a sustained release of dexamethasone [101]. Serendipitously, authors found that by increasing the dexamethasone content of the conjugate up to 24 wt%, the aqueous solution of the polymer conjugate became thermosensitive, changing from a fluid liquid at 4°C to a hydrogel at 30°C. Injection of the conjugate into a mouse model of osteoarthritis led to the formation of a hydrogel that was retained in the joint for more than a month, where it underwent progressive dissolution, releasing the water-soluble conjugate [102]. This release was, however, relatively slow (up to 7% per week), which is inappropriate for acute inflammatory conditions. To address this issue, Libánská *et al.* [103,104] developed an HPMA-dexamethasone conjugate through a pH-sensitive hydrazone linkage allowing rapid drug release at pH 5, mimicking the environment inside immune cells. In an *in vivo* mouse model of arthritis, a rapid accumulation of the conjugate within 15 minutes after its administration was observed in the inflamed tissues. Additionally, the severity of inflammation was reduced by more than 90%, compared to 40% for free dexamethasone showing the potential of this conjugate. Undesirable side effects could also be reduced with the HPMA conjugate, notably with a significant reduction of bone loss compared to the free drug [87].

Recently, a mechanistic study was performed by Jia *et al.* [91] to investigate further the impact of the linker chemical structure on the therapeutic efficacy of HPMA conjugates. Five HPMA copolymer-based dexamethasone conjugates were synthesized by adjusting the bond chemistry between the drug and the methacryloyl group (nature and position of the cleavable bond, the addition of a benzene ring or an amide to the linker, Fig. 7). A direct correlation between their therapeutic efficacy and the release rate was established. While faster conjugate activation provided a robust, immediate therapeutic response *in vivo*, the conjugate with C3 hydrazone linker (monomer E, Fig. 7), showing the slower release rate, was the most effective in preserving joint structural integrity.

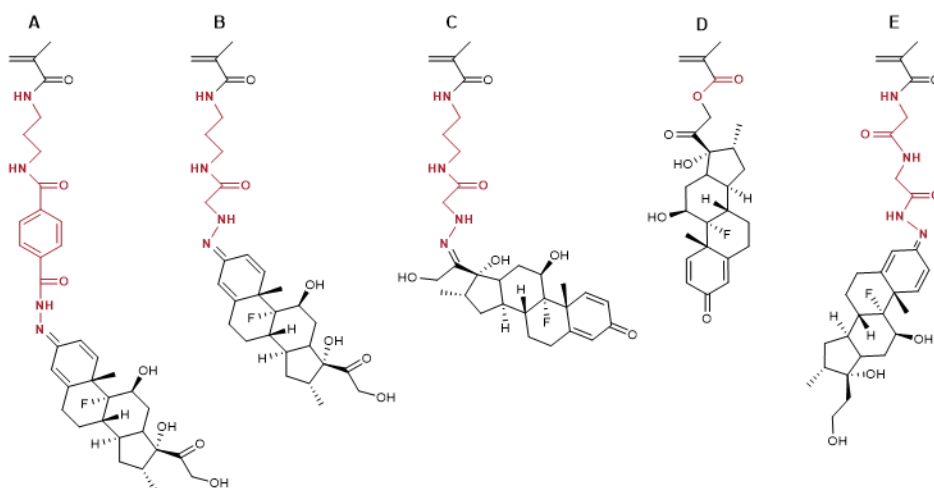


Fig. 7. Dexamethasone-containing monomers (A, B, C, D, E) for the synthesis of HPMA copolymer-based dexamethasone conjugates with different releasing rates. Red-colored structures represent the different linker chemistries. (from Jia *et. al.* 2020)[91].

The HPMA-dexamethasone conjugates were also successfully studied to treat inflammatory bowel disease after intraperitoneal administration by taking advantage of the ELVIS effect [105]. However, other colon-targeting conjugates orally administered may be more appropriate due to a more localized targeting and release approach reducing the risk of off-target or early systemic release [106–110].

Compared to the liposomal formulation [42,46,47], HPMA conjugates may be longer to synthesize and prepare. Still, they better control the drug release as the bond can only be hydrolyzed in an acidic environment. The main drawback of HPMA is its lack of biodegradability, which raises toxicity concerns due to body accumulation. However, it was shown that the polymer could be eliminated through glomerular filtration with a molar mass below 70 000 g/mol [95].

The release rate of the drug from the conjugate can be modulated by the type of polymer used, as shown by Funk *et al.* [111], who synthesized an α -methoxy- ω -poly(ethylene glycol) (AMPEG)-dexamethasone conjugate with the same acid-cleavable hydrazone bond. The drug becomes water-soluble by conjugating dexamethasone to an AMPEG polymer. It can be administered intravenously, allowing the conjugate to accumulate in inflammatory sites due to its large molar mass of 22 000 g/mol. It progressively released up to 62 % of dexamethasone *in vitro* over 14 days, much higher than the HPMA conjugate, which released only 15 % over the same period [92,99]. However, this PEG derivative conjugate can carry only one molecule of dexamethasone per polymer chain, resulting in a low drug loading of 0.63 wt%. Therefore, to reach the same dose of dexamethasone as HPMA conjugate, which carried 5 to 10.6 wt% of the drug, more polymer chains would have to be

administered, posing toxicity concerns considering the non-biodegradability of PEG and the controversy surrounding its biocompatibility [112,113].

To increase the loading capacity of PEG conjugates, an acetylene-modified, linear, and multifunctional click PEG has been synthesized by Liu *et al.* [114]. The conjugate synthesis was carried out by reacting a dexamethasone-containing monomer with an acetylene-terminated PEG and an acetylene mPEG. This reaction produced a conjugate carrying multiple dexamethasone molecules with a loading of 13.5 wt%. **Despite a slow release *in vitro* (9 % of the drug was released in the acidic medium after 17 days), the conjugate efficiently reduced inflammation *in vivo*.**

Natural polymers such as peptides or proteins have also been used as carriers for GC delivery. Taking advantage of discovering a series of cystine-dense peptides (CDPs) that rapidly accumulate in cartilage after systemic administration, Sangar *et al.* [115] have elaborated CDP conjugates carrying dexamethasone through a dimethyladipic acid-labile linker. Due to the small size of the peptides, a pretty high loading of around 10 wt% was achieved. *In vivo* studies have shown that the conjugate had favorable biodistribution and reduced systemic exposure compared to free dexamethasone. **Larger size proteins have also been employed for intra-cartilage delivery of GC. Bajpayee *et al.* [116] have conjugated dexamethasone to a highly basic protein, avidin (Mw ~66 kDa).** The introduction of an acid-cleavable hydrazone bond between the protein and dexamethasone slowed the release at pH 7.4 (30% in 350 h) and ensured a selective release in an acidic environment (80% in 150 h at pH 4). After intra-articular injection into an osteoarthritis model, the avidin-dexamethasone conjugate suppressed injury-induced joint inflammation and catabolic gene expression to a greater extent than free dexamethasone [117]. However, to deliver a sufficient amount of dexamethasone (0.5 mg), a high mass of avidin is required (20 mg), which is potentially cytotoxic. To avoid side effects, the authors have developed a multi-arm avidin containing 28 sites for covalent conjugation of drugs compared to the 4 sites in the previous design [118]. In addition, as shown in Fig. 8, succinic, dimethylglutaric, and phthalic anhydrides were used to form hydrolyzable ester linkers leading to conjugates with drug loading content of 15.7, 3.8, and 7.8%, respectively, compared to only ~ 2% with the original design. By varying the linker chemistry, the release rate could be modulated from rapid release (half-life of 6.8 h with the succinyl conjugate) to a prolonged release with half-time values of 79 and 86 h using dimethylglutaryl and phthalyl conjugates. For a more effective treatment, a combination of fast and slow-releasing conjugates has been used to formulate a system that enabled a 50% release of dexamethasone in 38.5 h, followed by a sustained release of the remaining drug for the next two weeks. On cartilage explant models of osteoarthritis, a single dose of this combination suppressed IL-1 α -induced GAG loss, cell

death, and inflammatory response more effectively than dexamethasone alone over two weeks.

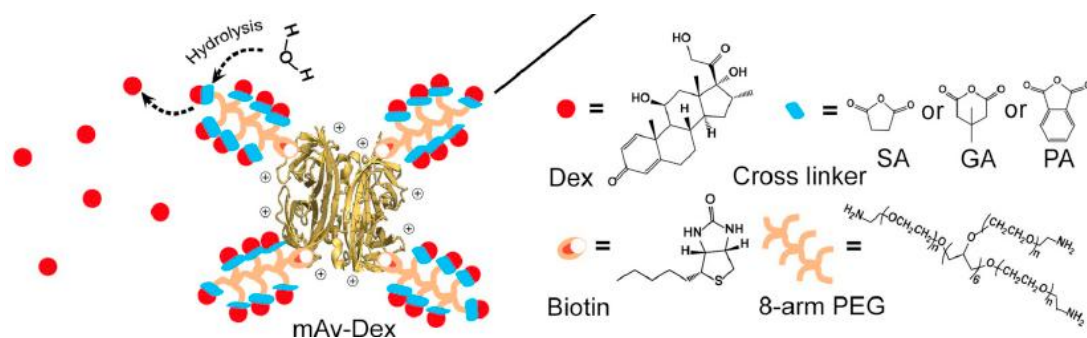


Fig. 8. multi-arm Avidin conjugated with dexamethasone (mAv-Dex) by using hydrolyzable ester linkers derived from succinic, glutaric, and phthalic anhydrides (SA, GA, PA) (from He *et al.* 2020)[118].

These pH-sensitive conjugates can also be helpful for other routes of administration, even though most are currently developed for systemic administration. Cao *et al.* [119] aimed to produce a conjugate coated onto electrodes for implantation in the cortex of patients with paralysis. Since the insertion of implants can cause injuries, an anti-inflammatory agent would be beneficial in limiting scarring. Therefore, a poly(N-vinyl pyrrolidone) (PNVP) polymer conjugate with prednisolone via a pH-sensitive hydrazone bond was synthesized and mixed with poly(acrylic acid) to form a coating on the electrode's surface. PNVP has proved safe as it is non-toxic and does not induce a pro-inflammatory reaction with macrophages. Even though it is not biodegradable, it can be excreted by the kidneys via glomerular filtration up to a molecular weight of around 50 000 g/mol [120]. **As expected with this type of bond, the amount of prednisolone released from the conjugate was higher at acidic pH than at physiological pH.** However, *in vitro*, the PNVP-prednisolone conjugates were less effective in reducing inflammation than the free drug. The authors hypothesize that depending on the conformations of the polymer chain, only a fraction of the conjugated drug molecules is accessible to the receptors [119].

The design of pH-sensitive conjugates offers a smart delivery strategy for localized GC release for inflammatory diseases. The drug release rate can be easily adjusted depending on the nature of the hydrolyzable linkage, from relatively fast release with acetal, imine, or hydrazine linkages to slow release with ester linkages. Several examples show that water-soluble polymers such as HPMA and PEG increase circulation time. Still, with the rapid development

Table 1. Hydrophilic polymer-glucocorticoid conjugates to treat inflammatory diseases.

Drug	Polymer	Mw (kg/mol)	Drug binding sites	Drug loading (wt %)	Type of bond/linker	Main type of release	Administration	Medical use	References
Dexamethasone	HPMA	73	Multiple	5-10.6	Hydrazone	pH-sensitive	IV	RA	[99]
Dexamethasone	HPMA	34-36	Multiple	9.2-10	Hydrazone	pH-sensitive	IV	RA	[87,92,97,100,101]
Dexamethasone	HPMA	36.8	Multiple	14	Hydrazone	pH-sensitive	IP	IBD	[105]
Dexamethasone	Acetylene PEG	17	Multiple	13.5	Hydrazone	pH-sensitive	IV	RA	[114]
Dexamethasone	Cystine peptide	~4	1	10	Dimethyl adipic	pH-sensitive	IV	RA	[115]
Dexamethasone	Avidin	~66	Multiple	2-16	Hydrazone	pH-sensitive	Cartilage explants	RA	[116–118]
Prednisolone	PNVP	17	Multiple	16	Hydrazone	pH-sensitive	Neural implant coating	Paralysis	[119]
Dexamethasone	AMPEG	22	1	0.63	Hydrazone	pH-sensitive	IV	RA	[111]
Dexamethasone	Dextran	72	Multiple	8.6	Succinate	Enzymatic	Oral	IBD	[108,109]
Dexamethasone				5.7	Glutarate				
Methylprednisolone				10.5	Succinate				
Dexamethasone	Dextran	70.4	Multiple	11	Succinate	Enzymatic	Oral	IBD	[110]
Methylprednisolone	Dextran	73	Multiple	8	Succinate	Chemical hydrolysis	IV	Liver transplant	[121,122]
Methylprednisolone	Dextran	25	Multiple	6.9-9.5	Succinate-Glycine (1-5)	Enzymatic and chemical hydrolysis	IV	Liver transplant	[123]
Budesonide	Dextran	10	Multiple	19.3	Succinate	Enzymatic	Oral	IBD	[124,125]
		70		14.3					
		500		11.6					
Budesonide	Dextran	10	Multiple	7.4	Glutarate	Enzymatic	Oral	IBD	[126]
		70		5.2					
		500		13.8					
Dexamethasone	pDMAEMA	9.78	Multiple	20-33	Acetate	Enzymatic	Oral	IBD	[106]
Dexamethasone	PLAA	30	Multiple	10	Ester	Enzymatic	Oral	IBD	[107]
Prednisolone	Glycol chitosan	-	Multiple	8.5	Succinate	Enzymatic and chemical hydrolysis	IV	RA	[127]
Fluocinolone acetonide	PEG	6	2	6.1	Succinate	Enzymatic and chemical hydrolysis	Local injection	Ocular diseases	[128]

HPMA: N-(2-hydroxypropyl)methacrylamide. **PEG:** polyethylene glycol. **PNVP:** poly(N-vinyl pyrrolidone). **AMPEG:** a-methoxy-w-amino-PEG. **pDMAEMA:** poly(dimethylamino) ethyl methacrylate. **PLAA:** poly(L-aspartic acid). **Mw:** molecular weight. **IV:** intravenous. **IP:** intraperitoneal. **RA:** rheumatoid arthritis. **IBD:** inflammatory bowel disease.

of polymer synthesis techniques, increasingly sophisticated multifunctional carriers are being developed that require careful attention to their biological fate after administration.

4.1.2 Enzymatically cleaved conjugates

Enzyme-sensitive linkers can be used to trigger the drug release at a specific site to improve the targeting potential of GC conjugates. As an example, many dextran-GC conjugates (Table 2) have been developed to treat inflammatory bowel diseases (IBD) such as ulcerative colitis or Crohn's disease, where the early release of the drug is an issue [109,110]. By conjugating dexamethasone or methylprednisolone to dextran through a succinate or glutarate linker, McLeod *et al.* synthesized conjugates that proved to be resistant to chemical hydrolysis at pH 6.8 and released only a tiny amount of the drug (< 3 %) when incubated with the small intestine contents of the rat. In contrast, the release was higher in the large intestine contents (14 % over 160 min), where dextranases and esterases of the gut microflora are abundant. Nevertheless, the GC release remains relatively low, probably because dextran sterically prevents the enzymatic hydrolysis of the linker ester and releases the drug only after bacterial degradation of the polymer backbone by dextranases [108]. *In vivo*, all the conjugates were more efficient in reducing inflammation in a model of colitis, with dexamethasone and the succinate linker providing the best results regarding colitis repair. It was also established that significantly less GC are transported in plasma by bypassing systemic absorption by the small intestine, thus avoiding undesirable side effects such as adrenal suppression [109].

The influence of dextran molecular weight on the efficacy of GC conjugates has also been studied for the treatment of IBD [124]. Synthesized budesonide-21-succinate was conjugated to dextran polymers with different molecular weights (10,000, 70,000, and 500,000 g/mol), producing conjugates with budesonide loading of 19.3, 14.3, and 11.6 wt%, respectively. The release studies showed that the high molecular weight dextran hindered the drug release in all intestinal segments due to higher chain entanglements which prevent the hydrolysis reaction. On the other hand, there was less protection from the small intestine esterases with the smallest polymer, resulting in a premature release of the drug. Conjugate prepared with dextran 70,000 g/mol was the most suitable size as it released twice as much budesonide hemisuccinate in large intestine contents compared to the two others. In addition, by changing the nature of the linker from succinate to glutarate, the release of budesonide was reduced by about half, demonstrating the impact of linker length on the properties of the conjugate [126]. These conjugates allowed a better healing of the colon and a significant decrease in inflammation as they avoided a premature release of the predominantly delivered drug throughout the colon [125,126].

Systems with a similar strategy to treat IBD have been developed with a variety of polymers such as poly(L-aspartic acid) (PLAA) [107] and poly(dimethylamino)ethyl methacrylate (pDMAEMA) (Fig. 6), which additionally has mucoadhesive properties [106]. These conjugates have also shown superior anti-inflammatory activity due to a sustained release in the large intestine. The large size of the polymers prevents premature hydrolysis of the ester bonds. It decreases the possibility of a passage through the epithelial cells to the systemic circulation, thus reducing the risk of systemic exposure to GC.

In the same way that dextran protects the ester bonds in the small intestine, it also prevents early drug release in blood for conjugates administered by the IV route [121,122]. Methylprednisolone linked to a 70,000 g/mol dextran through a succinate linker was developed to achieve targeted and sustained release in a transplanted liver through the ELVIS effect to prevent graft rejection. **The resulting dextran conjugates were resistant to degradation in the bloodstream and also avoided the systemic release of GC. Unfortunately, they were highly resistant to degradation by liver lysosomes due to steric hindrance limiting esterase access.** A smaller 25,000 g/mol dextran was used to address this issue, and peptides of different lengths were introduced into the linker between methylprednisolone succinate and dextran. *In vitro* studies have shown that the release rate of the drug was proportional to the length of the peptide linker. The authors attributed this to the fact that longer linkers have more electron-withdrawing groups due to their multiple amide bonds. Hence, they become more polarized and more sensitive to nucleophilic attack by water [123]. This trend was confirmed *in vivo* with a higher release rate with conjugate containing five amino acids as linker than the one containing only one amino acid [129,130]. The conjugates were all sufficiently stable in blood, allowing a hepatic accumulation, and were able to release methylprednisolone progressively in the presence of lysosomal enzymes. A right balance between the stability and degradability of polymers and their linkers is required to allow the proper release of the drug.

Apart from inflammatory bowel diseases, enzymatically-cleaved conjugates have also been developed for the intra-articular delivery of GC. Formica *et al.* [131] designed and synthesized a cartilage-specific conjugate by linking dexamethasone to a deacetylated chitosan (deacetylation degree of 88%, Mw 100-200,000 g/mol) via a two-step reaction (Fig. 9). First, dexamethasone was methacrylated on carbon-21 and then coupled to the chitosan through these amine functions. They also synthesized a second conjugate using a collagen type II-binding peptide by Steglich esterification. A glycine linker was added for steric reasons (Fig. 9B). Both conjugates were able to release active dexamethasone by ester linkage hydrolysis in the presence of an enzyme. An *in vitro* study on a bovine explant model

showed that the two conjugates exhibited prolonged drug retention within the cartilage, resulting in enhanced anti-inflammatory effects compared to free dexamethasone.

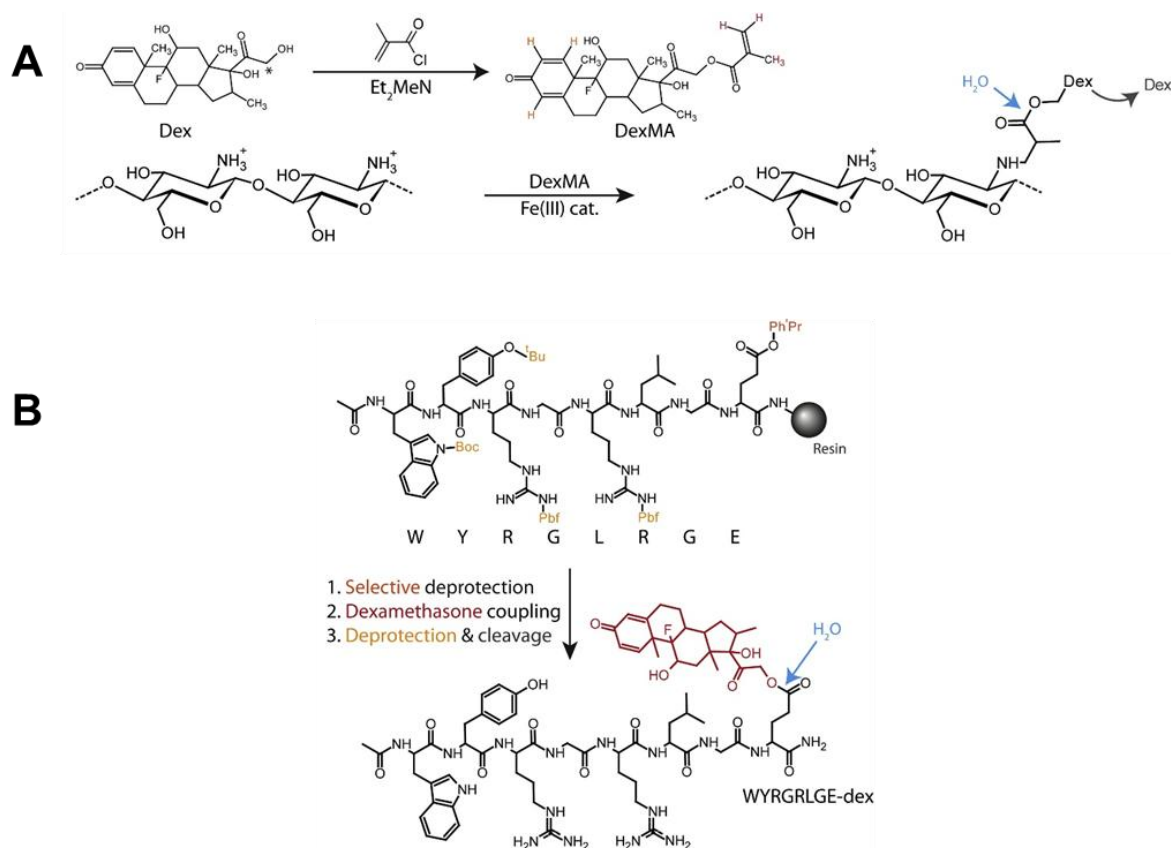


Fig. 9. (a) Scheme of the synthesis of a chitosan-dexamethasone conjugate, (b) Scheme of the synthesis of dexamethasone-collagen type II-binding peptide conjugate (from Formica *et al.* 2019) [131].

Conjugates with other polymers displaying linkers sensitive to enzymatic degradation have been produced for different diseases such as PEG-fluocinolone acetonide to treat ocular inflammation locally by ensuring a progressive release of the GC thus reducing the frequency of administration. PLGA nanoparticles encapsulating the same drug were formulated as a comparison, and while both formulations were therapeutically effective, the conjugate avoided an initial burst release of the drug [128].

Dendrimers are other attractive polymer candidates for drug conjugates due to their well-defined branched structure and high density of surface functional groups for chemical modification. Poly(amidoamine) (PAMAM) dendrimers have been frequently studied to deliver various glucocorticoids in lung and ocular inflammation. Along this line, Inapagolla *et al.* have conjugated methylprednisolone onto the generation 4 (G4) PAMAM-OH dendrimer to treat lung inflammation associated with asthma [132]. **To facilitate conjugation, a spacer was first linked to methylprednisolone before reacting with the PAMAM dendrimer (Fig. 10). In this way, 12 molecules of methylprednisolone per dendrimer could be introduced leading**

to a loading of ~32 wt%. Perumal *et al.* [133] have shown that the anti-inflammatory activity of these dendrimer conjugates was influenced by the type of spacer, particularly glutaric acid showed significantly lower activity than the conjugate formed from succinic acid, which possesses one supplementary carbon on its structure. The *In vivo* efficacy of the glutaric acid G4-PAMAM conjugate in a mouse lung inflammation model has been shown to enhance the ability of the methylprednisolone to inhibit inflammation by prolonging the residence time of the drug in the lung [132].

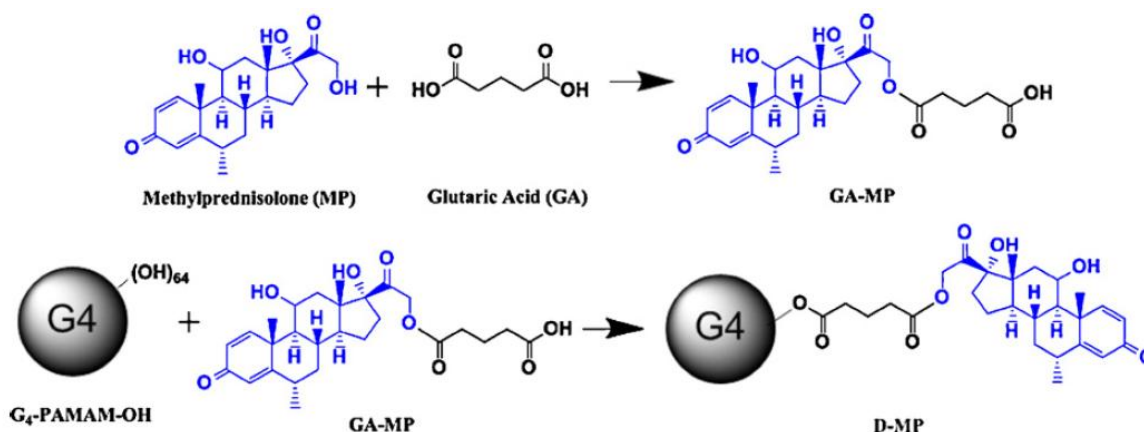


Fig. 10. Synthesis scheme of PAMAM-G4-OH-MP conjugate. From [132]

Dendrimer-dexamethasone conjugates using different PAMAM generations have been produced by Yavuz *et al.* [134] to treat an ocular disease affecting the retina. The drug was directly linked to the PAMAM-COOH through an esterification reaction in the presence of DCC and DMAP. Around 10 and 4.5 molecules of dexamethasone per dendrimer were conjugated to (G3.5) PAMAM and (G4.5) PAMAM, respectively. The release behavior performed in the presence of ocular esterase enzymes was very low. Less than 8% of dexamethasone was released in 6 days for (G3.5) PAMAM conjugate and around 2% for (G4.5) PAMAM conjugate. Despite these long release times, *in vivo* studies performed on rats have shown that both conjugates could increase the amount of dexamethasone reaching the retina after subconjunctival injection.

Kambhampati *et al.* [135] have also produced G4-PAMAM dendrimer-triamcinolone acetonide (TA) conjugate to improve permeation through ocular tissue and intracellular transport. The conjugation of triamcinolone acetonide-21-glutarate with the hydroxyl groups of the dendrimer resulted in dendritic conjugate with 10 molecules of TA conjugated per dendrimer (~21 wt%). The toxicity profile on microglial and human retinal pigment epithelium cells was significantly improved compared to free TA. Release in a simulated vitreous humor solution at pH 7.0 led to an initial burst release of ~18 wt% and then the conjugate exhibited sustained release of TA ~2% per day for 45 days. *In vitro* study showed that the conjugate

was ~100-fold more effective than free TA in suppressing lipopolysaccharide-induced inflammatory activity in cultured microglia and ARPE-19 cells. Recently, an *in vivo* study in a mouse model of oxygen-induced retinopathy demonstrated a significant superiority of these TA conjugates over free TA in suppressing retinal neovascularization and providing retinal neuroprotection [136].

In summary, these studies highlight the potential of enzyme-responsive delivery systems as an intelligent platform with site-specific drug release behavior. However, steric effects due to the entanglement of the polymer chains may impede enzyme access to the labile linkage, preventing the cleavage reaction and drug release. In a general way, polymer-drug conjugates have become an attractive and effective approach to improving therapeutic effects. Nevertheless, the relatively low drug loading for hydrophobic drugs as GC remains challenging.

4.2 Amphiphilic conjugates or nanoconjugates

Continuous improvements by directly conjugating a hydrophobic drug to a hydrophilic polymer having multiple conjugation sites have recently been significantly reported [137–139]. These amphiphilic conjugates can be synthesized with defined chemical structures and high drug-loading efficiency. In water, they self-assemble to form spherical nanocarriers such as micelles or nanoparticles composed exclusively of conjugates, thus forming "nanoconjugates" with the hydrophilic moieties turned outwards and the lipophilic elements inwards (Fig. 11, Table 3). These nanoconjugates can easily be administered as colloidal objects.

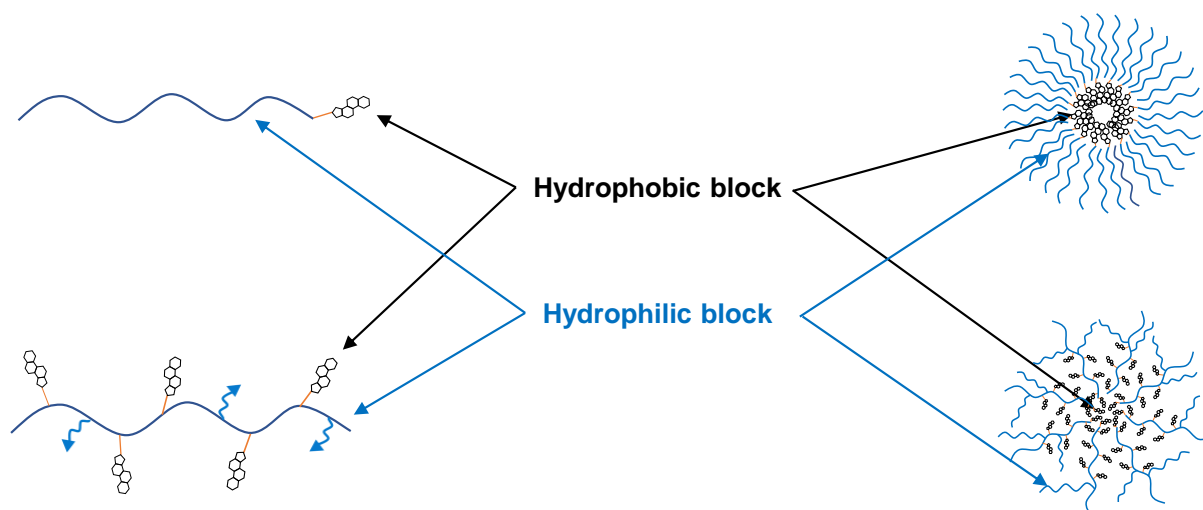


Fig. 11. Polymeric micelles or nanoconjugates made from drug-polymer conjugates.

Compared to drug physical encapsulation in nanoparticles, polymer-drug nanoconjugates show more advantages in improving stability, increasing drug loading, and limiting burst release [128]. Since GC are less accessible and protected from low concentrations of

esterases, they reduce the amount of non-specific interactions with potential blood components. By increasing the stability of the carrier, its half-life, and therefore the therapeutic effect of the associated drug can be prolonged.

4.2.1 Poly(ethylene glycol)-based nanoconjugates

As shown in Fig. 12, the most frequent polymers used to produce conjugate with GC are PEG derivatives or copolymers incorporating PEG. PE is a commonly used polymer that provides stealth properties to nanocarriers and, therefore, is an excellent candidate for producing nanoconjugates capable of avoiding opsonization following IV administration. Compared to the PEG used for hydrophilic conjugates (Table 2), those used to formulate nanoconjugates have lower molecular weights (Table 3). The resulting polymer will therefore become amphiphilic by binding a hydrophobic GC, allowing it to self-assemble into a spherical nanocarrier.

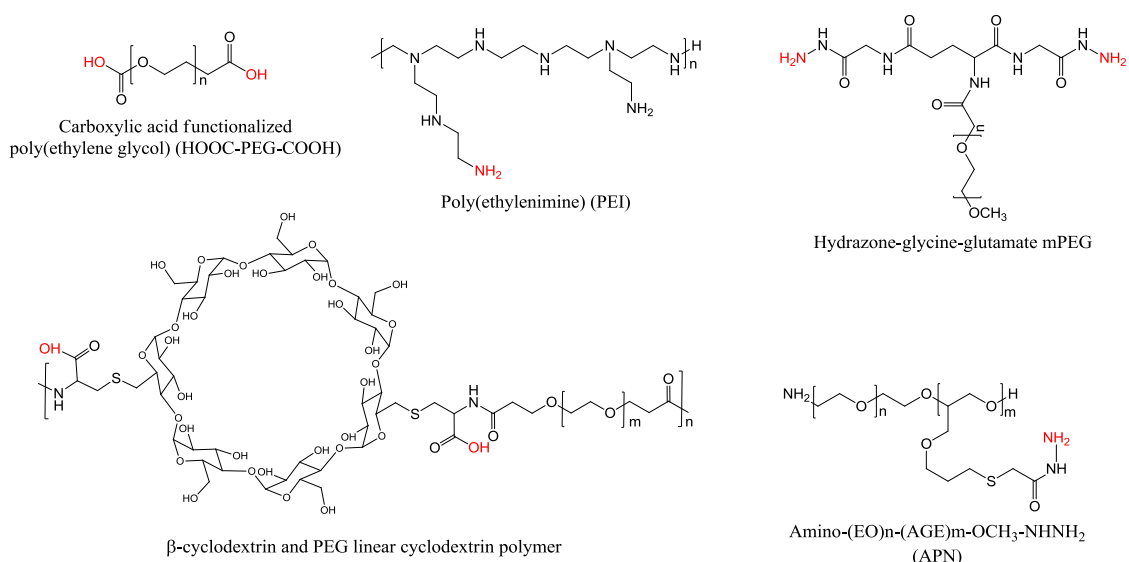


Fig. 12. Examples of polymers used for conjugation with glucocorticoids to produce amphiphilic nanoconjugates capable to self-assemble.

A conjugate of dexamethasone bound to a methylated PEG (mPEG), which can self-assemble into a micellar structure in aqueous media, was developed as a drug delivery vehicle to treat inflammatory and autoimmune diseases [140,141]. Two dexamethasone molecules were linked to a mPEG chain through a hydrazone-glycine-glutamate linker on dexamethasone's C-3 carbonyl group. The resulting nanoconjugate was able to accumulate in kidneys through the ELVIS effect and was taken up through endocytosis by inflamed renal cells. **In these cells, dexamethasone is released into the lysosomes where the hydrazone linker is cleaved by acidic pH.** The therapeutic effects were studied in mice with nephritis, where two monthly micelle IV injections were compared to the daily administration of free dexamethasone by the same route. The nanoconjugates showed superior and sustained

therapeutic efficacy without the side effects commonly attributed to GC. A five-month long-term treatment study confirmed that the design of these nanoconjugates with high tropism for kidneys significantly modified the pharmacology of dexamethasone, resulting in localized and long-lasting anti-inflammatory and immunosuppressive effects without triggering any measurable GC-related side effects [142].

Although hydrazone is the most used labile bond for the conjugation of drugs, other labile bonds, such as disulfide have been reported. Tran Thi *et al.* [143] used activated mPEG for coupling with cystamine dihydrochloride and in this way, introduced a biarm structure with a bioreducible disulfide bond. Afterward, dexamethasone was coupled to the modified mPEG via carbodiimide chemistry to produce a conjugate with 23 wt% of dexamethasone. Micelles with sizes in the range of 130-150 nm were produced, showing good colloidal stability. In the presence of dithiothreitol (10mM), a reducing agent, micelles underwent rapid destabilization within 3 hours, while no significant change was observed with the nonreducible conjugates. This result demonstrates the potential of this conjugate to formulate an efficient nanocarrier for the intracellular targeted delivery of drugs.

A polymeric micelle composed of an amino-PEG derivative carrying prednisolone through a hydrazone bond was developed by Li *et al.* [16]. A cationic branched PEG polymer (denominated APN) was first synthesized before adding a hydrazone linker to allow the conjugation of prednisolone with a drug loading rate of 19.3 wt%. As expected, drug release was much faster in acidic pH (5.5 or 6.0), with 60 % of the load released within 20 hours, when at physiological pH (7.4), less than 40 % of the drug was released after 96 hours. *In vivo*, micelles were stable in blood, and they selectively accumulated in inflamed joints of mice with arthritis delivering a higher dose of prednisolone than the free drug. Prednisolone was released from APN in the acidic environment of synovial fluid. It effectively reduced inflammation while preserving bone density and avoiding other known GC side effects such as hepatotoxicity and hyperglycemia. Recently, this research group extended their study by attaching dexamethasone instead of prednisolone to the amino-PEG derivative [144]. Three PEG conjugates with different dexamethasone content (30, 79, and 120 µg/mL) were synthesized to form pH-sensitive micelles. Among these, the micelle with the highest dexamethasone density showed superior *in vivo* efficacy with the longest circulation time and highest drug accumulation in inflamed joints. An increase in the grafted amount of dexamethasone on the polymer (165 µg/mL) did not allow the conjugate to self-assemble in an aqueous solution due to a very high hydrophobicity.

Hwang *et al.* synthesized a glycinate derivative of methylprednisolone and then conjugated it to a linear cyclodextrin polymer of 117,000 g/mol composed of β-cyclodextrin and PEG,

producing a conjugate with a 12.4 wt% loading rate [145]. In water, it self-assembles into nanoconjugates with a diameter of 27 nm. In PBS, the half-life of released methylprednisolone was 50 hours. In contrast, it was shortened to 19 hours in plasma, most probably due to the presence of esterases accelerating the hydrolysis of the conjugate's ester bonds. This half-life is still much longer than free methylprednisolone, proving that the nanoconjugate could decrease the clearance and increase the drug's residence time in circulation, allowing it to accumulate in inflamed joints. After releasing the drug, nanoconjugates disassembled into polymer strands, showing the role of lipophilic methylprednisolone in the self-assembly process of the conjugate. In *in vivo* tests on collagen-induced arthritic mice, nanoconjugates administered weekly at up to a 10-fold lower dose were as effective in reducing the arthritic score as daily administration of free methylprednisolone dose at an equivalent dose after 28 days.

To improve the uptake of the nanoconjugate by the target cells, Hu *et al.* [146] conjugated sialic acid in addition to GC onto the polymer. In this way, they produced sialic acid-PEG-dexamethasone conjugates capable of self-assembling into micelles while encapsulating additional free dexamethasone for intra-articular administration. The conjugate is formed through an esterification reaction between the carboxylic functions of a modified PEG and the hydroxyl groups on dexamethasone and sialic acid. It comprises a 2,000 g/mol carboxylic acid-functionalized PEG chain with conjugated dexamethasone at one end and sialic acid at the other end, leading to a dexamethasone content of about 16 wt%. This conjugate was then formulated into micelles through a solvent diffusion method using its amphiphilic properties to spontaneously self-assemble in aqueous media. Free dexamethasone was also encapsulated by physical entrapment during the formulation of the micelles with a drug loading of 6.3 wt% in addition to the amount already conjugated on the polymer. *In vitro*, micelles released a large amount of dexamethasone in the first 12 hours and sustained the drug's release over 48 hours. This profile demonstrates the biphasic release with an initial phase corresponding to the physically entrapped dexamethasone followed by the conjugated one. In addition, a faster cellular internalization of micelles conjugated with sialic acid was observed compared to those composed of PEG-dexamethasone only, showing the efficacy of targeting in the presence of sialic acid. Targeting was also beneficial in *in vivo* experiments where micelles with sialic acid accumulated more than those without the ligand in the kidneys and had a more substantial sustained anti-inflammatory effect.

As seen above, PEG is an excellent candidate for drug conjugation due to its unique characteristics *i.e.* such as low toxicity, biocompatibility, and inert nature. While there are certain limitations associated with PEG such as its non-biodegradability, structural

modifications in PEG can overcome these limitations. However, although PEG was considered a non-immunogenic molecule for a long time, as suggested above, there is growing evidence that PEG is immunogenic [77], so attention should be paid when using it in pharmaceutical formulations.

4.2.2 Poly(ethylenimine)-based nanoconjugates for gene delivery

Safe and effective gene delivery has proven challenging, notably with recombinant viral vectors, so nonviral alternatives have been considered. Nanotechnologies are gaining interest in gene delivery as they have lower immunogenicity than viral vectors. However, their transfection efficiency is lower [147]. Therefore, new GC systems have recently been investigated to take advantage of their translocation to the nucleus after binding to the GC receptor. By conjugating GC to a polymer and formulating a nanoconjugate on which DNA can be transported, translocation of the entire nanocarrier into the nucleus via the GC-GC receptor system could be ensured. In addition, GC would inhibit the production of pro-inflammatory cytokines that occurs after gene delivery [148].

Various cationic polymers, such as poly-L-lysine, chitosan, and polyethylenimine (PEI), have been used in pharmaceutical and biological studies, with PEI being the most promising. It is a cationic polymer containing numerous amino groups with high positive charge density and high chain flexibility. This allows it to self-assemble easily with negatively charged nucleic acids through electrostatic interaction, thus significantly improving their transfection efficiency. A novel gene delivery system combining dexamethasone and poly(ethylenimine) (PEI) has proven to be efficient in the delivery of plasmid DNA (pDNA) in a model of acute lung injury to release genes that reduce hypertension, edema, and alveolar inflammation [149]. Dexamethasone has a dual role in this nanoconjugate system. It serves as a translocation vehicle by binding to the glucocorticoid receptor, bringing pDNA into the nucleus, and reducing inflammation through its therapeutic activity. A dexamethasone-21-mesylate derivative was conjugated with a 2,000 g/mol branched PEI and then complexed with pDNA forming a micellar structure. The nanoconjugates formed stable structures protecting pDNA from degradation in plasma. They had a higher transfection efficiency than PEI without dexamethasone and successfully delivered genes to the lungs *in vivo* while also inducing an anti-inflammatory action [149].

Similarly, Yi *et. al.* have also produced dexamethasone-PEI nanoconjugates harboring pDNA with chondrogenesis factors to induce chondrogenic differentiation of mesenchymal stem cells to repair cartilage defects. Dexamethasone and tetramethylrhodamine for imaging purposes were conjugated to a branched PEI of 25,000 g/mol. The nanoconjugate successfully transported the pDNA into the nucleus of cells *in vitro*, as evidenced by the

chondrogenic differentiation obtained, in a more efficient manner than PEI alone and with lower cytotoxicity towards cells [150].

Dexamethasone-PEI nanoconjugates have also been encapsulated in DOTAP/cholesterol liposomes to improve transfection efficiency compared to the nanoconjugate alone on the neuroblastoma cell line. Indeed, these lipopolyplexes appeared to be better suited to escape endosomal and lysosomal degradation after cell endocytosis, allowing the system to be translocated into the nucleus without degrading the transported pDNA [151]. While lysosomal degradation would be desirable to release GC for anti-inflammatory purposes, in the case of gene delivery, this should be avoided to achieve the goal of gene transfection.

Su *et al* developed an alternative to the use of polyethyleneimine as a hydrophilic polymer carrier for the treatment of acute lung inflammation [152]. To achieve proper targeting of alveolar macrophages which play a key role in inflammation, the authors introduced mannose into pH-sensitive dexamethasone nanoconjugates. After grafting dexamethasone onto a 2,000 g/mol branched PEI via a pH-sensitive Schiff base linkage, the protected mannose-functionalized cyclic carbonate was introduced by a simple click reaction generating a carbamate linkage. The obtained nanoconjugates with a high drug loading of 52 wt% showed significantly enhanced cellular uptake by macrophages, with a 2.23-fold higher uptake than the non-targeted nanoconjugates. These results were confirmed *in vivo* in a mouse model where a significantly higher accumulation of nanoconjugates was observed in the lungs.

PEI seems to be an efficient vehicle on which GC can be conjugated to form nanocarrier complexes with DNA for gene delivery. However, the molecular weight of this polymer must be chosen with care as it can be toxic at higher molar masses. It should remain between 5,000 and 25,000 g/mol, ensuring suitable gene transfer efficacy while reducing toxicity [153].

4.2.3 Polysaccharides-based nanoconjugates

Biodegradable natural polymers such as polysaccharides have often been used as carriers to formulate nanoconjugates since they possess many chemical functional groups on their backbone for conjugation with drug molecules. Moreover, they are naturally abundant and available, remarkably biodegradable and biocompatible, and generally have low toxicity, which is favorable for drug delivery systems. Chitosan is a linear polysaccharide with functional amino groups that can be modified with hydrophobic moieties for direct self-assembly into nanoconjugates. Based on this functionality, an intravitreal glucocorticoid delivery system was developed by Dubashynskaya *et al.* [154] to treat inflammatory diseases of the posterior segment of the eye. A two-step synthesis allowed the preparation

of chitosan-succinyl-dexamethasone nanoconjugates with different degrees of substitution of dexamethasone moieties from 2 to 4%. These amphiphilic species are self-assembling into 400-1200 nm nanoconjugates. The release at pH 7.4 displayed a slow hydrolysis rate with a total release of 8–10% for dexamethasone and succinyl dexamethasone after 1 month. In TNF and LPS-induced inflammation models, the developed nanoconjugate with the highest degree of substitution has shown a significant anti-inflammatory effect, suppressing CD54 expression in THP-1 cells by 2 and 4-fold, respectively. Similar nanoconjugates with a degree of substitution up to 5.75% have shown effective ocular tolerance *in vivo* and provided longer precorneal retention compared with an aqueous formulation, showing the promise of this ophthalmic drug delivery system [155].

Heparin is a highly sulfated polysaccharide, which confers a negatively charged property, favoring electrostatic interaction with several proteins. In particular, binding to a serine protease inhibitor or anti-thrombin promotes its selectivity and specificity toward cancer cells. In light of this benefit, heparin has often been used to develop carriers for chemotherapeutic drugs. Li *et al.* [156] synthesized heparin nanoconjugates to co-deliver dexamethasone and doxorubicin to initially treat multiple myeloma. First, different amounts of dexamethasone were conjugated to heparin through an acid-labile hydrazone linkage, forming amphiphilic macromolecules. These macromolecules were then able to self-assemble to form nanoconjugates containing 6-13 wt% dexamethasone, in which doxorubicin was physically encapsulated into the hydrophobic core (~19 wt%). As expected, the release behavior of both drugs from these nanoconjugates was pH-dependent with only 3% of dexamethasone and 7% of doxorubicin released at pH 7.4 after 3 days while more than 18 % of dexamethasone and 23% of doxorubicin were released at pH 5. Furthermore, *in vitro* experiments on human nasopharyngeal carcinoma CNE1 cells showed that these nanoconjugates were easily internalized and the drug accumulated in the tumor cells. This phenomenon was attributed to the nucleus-targeting ability of conjugated dexamethasone on heparin.

To improve the cytotoxic effect through the synergistic effect of the combination of anti-inflammatory and anti-cancer drugs, D'arrigo *et al.* [157] have developed a novel type of gellan nanoconjugate system for carrying physically-entrapped paclitaxel and chemically linked prednisolone. Direct grafting of prednisolone onto the native gellan gum was not possible, probably due to the polymer chains' stiffness and high molecular weight. Therefore, in a preliminary step, the molar mass of the gellan gum was reduced by ultrasound and a short spacer was introduced onto prednisolone by esterifying the hydroxyl group on C21 with 4-bromobutyric acid to prevent the steric hindrance induced by the polysaccharide [158]. Subsequently, the brominated prednisolone was grafted onto the low molar mass gellan gum

to self-assemble and form nanoconjugates containing 6% of prednisolone with a size of ~280 nm. Paclitaxel was then physically entrapped by simple hydration of a dry film of the anti-cancer drug by an aqueous solution of nanoconjugates to yield a drug loading of 7 wt%. *In vitro* tests have shown that prednisolone released by the nanoconjugates significantly suppressed the production of TNF- α in LPS-stimulated cells. Moreover, enhanced cytotoxicity on various cancer cell lines was achieved with these prednisolone-paclitaxel nanoconjugates compared to the free prednisolone and similar nanoconjugates grafted with cholesterol instead of prednisolone. Thus, this dual-drug nanoconjugate system could be helpful in cancer therapy.

Conjugation of GC to polysaccharides is another promising strategy, especially since many polysaccharides are biodegradable, have low toxicity, and generally do not induce a strong immune response. However, the direct conjugation of drugs to the amino, carboxyl or hydroxyl groups of polysaccharides can induce a structural change in the polymer chain and functional integrity.

4.2.4 Miscellaneous polymer-based nanoconjugates

Many other hydrophilic polymer structures have been used as drug carriers, such as polymethacrylates [159,160], polyacrylamides [161,162], poly(vinyl pyrrolidone) [119], and synthetic polypeptides [163,164]. These latter are particularly attractive as polymer vehicles due to their good biocompatibility, biodegradability, and functionalization [165]. Poly(L-lysine) based micelles have recently been prepared by direct conjugation of dexamethasone to the carboxylic acid side groups of the polymer chain for the treatment of colorectal cancer. Because of the presence of a disulfide linker [163], the release behavior of dexamethasone was redox-dependent with less than 10% of dexamethasone released after 120 h from the micelles without a sensitive linker while more than 66% was released with 10 mM of glutathione (a reducing agent). Biodistribution after intravenous injection in mice bearing CT26 tumors showed a significantly higher accumulation of dexamethasone in tumors in the sensitive micelles group than in the free dexamethasone group (9.7-fold at 48h hours). In addition, superior antitumor activity was demonstrated with a tumor suppression rate of ~86% compared to 49% in the free dexamethasone group. In another study, a pH-responsive nanoconjugate from poly-L-glutamic acid and fluocinolone acetonide was developed for the topical treatment of psoriasis [164]. The drug was connected to the polymer through the multifunctional carboxylic acid backbone to reach 23.8 wt% of fluocinolone. In water, the amphiphilic system self-assembles to produce nano-objects with a size of around 90 nm. Only 2.5 and 5.5% of fluocinolone were released from the nanoconjugates after 72h at pH 5.5 and 7.4, respectively. The presence of cathepsin B (a

lysosomal protease) significantly accelerates the release with 20% drug release within 72h. This was ascribed to the degradation of the polypeptide which facilitates the accessibility to the polymer-drug ester bond. To enhance skin permeation, the nanoconjugates were incorporated in a hyaluronic acid-poly-L-glutamate hydrogel and evaluated *in vivo* on a psoriasis mouse model. An 8.5-fold and 1.4-fold reduction in tissue concentrations of INF- γ and IL-23 was reported after treatment compared to the free drug, as well as a significant improvement in psoriatic condition.

For ocular therapy, a thermosensitive triblock N-isopropylacrylamide copolymer drug conjugate containing 6.3 wt% of dexamethasone was synthesized by RAFT polymerization [162]. The conjugate was crosslinked in presence of cystamine which reacted with the N-hydroxysuccinimide functionality of the polymer to form a hydrogel. The release of dexamethasone from the hydrogel occurred through ester hydrolysis for more than a year showing the potential of this system for treating chronic ocular inflammatory diseases.

Due to the overexpressed ROS levels in inflammatory regions, designing ROS-responsive materials as GC carriers may provide additional synergetic effects in inflammation treatment. Ma *et al.* [160] reported the synthesis of a theranostic nanoplatform with lipid-specific imaging and ROS responsiveness for the treatment of atherosclerosis. A commonly used glucocorticoid, prednisolone, was first linked to hydroxyethyl methacrylate via a ROS-sensitive linkage. Then, the copolymer was synthesized by RAFT polymerization with 2-methacryloyloxyethyl phosphorylcholine, 2-methylthio ethanol methacrylate and the prednisolone monomer. In PBS, the prepared copolymer possessed 10.7% of prednisolone, and its self-assembly by dialysis in PBS produced micelles with lipid-specific fluorophore encapsulated in their core. The typical pathophysiological condition of the inflammation site allows the micelles to accumulate at the level of inflammatory atherosclerotic tissue with overexpressed ROS. In presence of 1 mM H₂O₂ mimicking the oxidative stress in atherosclerotic tissue, 57 and 92% of prednisolone were released from micelles after 4 and 48 h, whereas less than 10% was released without H₂O₂ demonstrating ROS-dependent drug release. *In vivo* studies on mice ApoE^{-/-} showed that the encapsulated fluorophore can specifically combine with the accumulated lipid of atherosclerotic lesions with distinct green fluorescence and that the released prednisolone can achieve the effective anti-inflammatory activity. This platform opens promising perspectives for atherosclerosis theranostics.

In summary, various strategies are being developed to improve the efficacy of anti-inflammatory drugs as evidenced by the variety of polymers and stimuli-sensitive linkers used. This has led recently to the design of carriers with multiple functions such as high

circulation time, enhanced biological interaction, and *in vivo* traceability. The future challenge now will be to validate these systems in terms of safety and efficacy in humans.

Table 2: Amphiphilic polymer-glucocorticoid nanoconjugates.

Drug	Polymer	Mw (kg/mol)	Drug binding sites	Type of bond/linker	Type of release	Drug loading (wt %)	Size (nm)	ZP (mV)	Administration	Medical use	References
Dexamethasone	mPEG-COOH	3.1	2	Hydrazone	pH-sensitive	26.4	33	~0	IV	Lupus nephritis	[140,141]
Dexamethasone	mPEG-COOH	2	2	Disulfide	ROS-responsive	23	130			Cancer	[143]
Prednisolone	APN	5.1	Multiple	Hydrazone	pH-sensitive	19.3	45.8	2	IV	Rheumatoid arthritis	[16]
Methylprednisolone	β -cyclodextrin-PEG	117	Multiple	Glycinate	Enzymatic and chemical hydrolysis	12.4	27	-	IV	Rheumatoid arthritis	[145]
Dexamethasone	HOOC-PEG-COOH	2.7	2	Ester	Enzymatic and chemical hydrolysis	6.3	45	-13.3	IV	Acute kidney injury	[146]
Dexamethasone	bPEI	2.6	Multiple	Mesylate	-	15	100	30-45	IT	Acute lung injury, gene delivery	[149]
Dexamethasone	bPEI	25	Multiple	Succinate	-	-	139	33	-	Gene delivery	[150]
Dexamethasone	bPEI	3.8	Multiple	Mesylate	-	~42 (DS=9.7%)	120	47	-	Gene delivery	[151]
		26.3				~49 (DS=14.3%)	200	42			
Dexamethasone	bPEI	2	Multiple	Schiff base	pH-sensitive	52	115	31	IV	Acute lung injury	[152]
Dexamethasone	Chitosan	37	Multiple	Succinate	pH-sensitive	2-4	400-1200	~30	II	Glaucoma	[154,155]
Dexamethasone	Heparin	-	Multiple	Hydrazone	pH-sensitive	6-13	150	-	IV	Multiple myeloma	[156]
Prednisolone	Gellan gum	17	Multiple	Ester	Enzymatic and chemical hydrolysis	6	280	-20	IV	Cancer	[157]
Dexamethasone	Poly(L-lysine)	-	Multiple	Ester and disulfide	pH-sensitive and ROS responsive	17.1	33	-5.2	IV	Cancer	[163]
Fluocinolone acetonide	Poly-L-glutamic acid	15	Multiple	Ester	pH-sensitive	23.8	90	~50	Topic	Psoriasis	[164]
Dexamethasone	PNIPAM	37	Multiple	Ester and thioether	Chemical hydrolysis	6.3	-	-	Ocular injection	Ocular diseases	[162]
Prednisolone	PMM	23	Multiple	oxalate	ROS Sensitive	10.7	57	-	IV	atherosclerosis	[160]

PEG: polyethylene glycol. **APN:** Amino-poly(ethylene oxide)-poly(allyl glycidyl ether)m-OCH₃-NHNH₂. **bPEI:** branched poly(ethylenimine). **PNIPAM:** poly(N-isopropylacrylamide). **PMM:** poly(methacryloyloxyethyl phosphorylcholine).

MM: molar mass. **ZP:** zeta potential. **IV:** intravenous. **IT:** intratracheal. **II:** intravitreal. **DS:** degree of substitution.

5 Conclusion

NLP, polymer-drug conjugates, and nanoconjugates have proven to be promising alternatives to improve the therapeutic potential of GC and their prodrugs. These systems have shown their utility by increasing the solubility of GC, reducing their clearance, prolonging the drug residence time in circulation, and reducing toxicity by modifying various physicochemical properties. These properties govern the nanocarrier's stability and ability to accumulate in areas of inflammation by increasing their half-life and prolonging their effect or by using ligands for active targeting of specific cells.

GC NLP are highly stable and easy to produce by preparation methods that can be scaled up without any obstacles. However, they can only be obtained with the addition of stabilizing amphiphilic molecules such as PEGylated lipids or poloxamers. Although being used in liposomes or lipid nanoparticle formulation, excipients such as PEGylated lipids might induce an undesirable immune response. Future challenges would be to produce NLP with optimal long-circulating properties that do not include in their composition additional excipients and display more controllable drug-release kinetics. One option could be to test different types of lipid moieties and different kinds of linkages instead of the conventional ester bond.

Polymer therapeutics have provided novel carriers for GC, either as a water-soluble conjugate or a self-assembled amphiphilic nanoconjugate, with various options in types of polymers and linkers. The addition of linkers brought new functionality to these nanocarriers, which is the targeted release of the drug in a specific environment, either through the acidic pH of synovial fluid or enzymatic cleavage by specific esterases in the colon microflora or lysosomal content. The ideal linker should be stable in conditions outside the inflammation site and not sensitive to chemical hydrolysis at physiological pH.

Compared to conventional nanocarriers, polymer-drug conjugates are considered new chemical entities due to the covalent bond linking GC to the polymer and not just a new formulation of an existing GC. Thus, there may be regulatory hurdles to overcome and require in-depth toxicological studies. The polymers' biological effects and clearance and metabolites should be investigated.

Most polymers used for therapeutics, such as PEG and HPMA, are not readily biodegradable. So there is a need to develop more biodegradable and biocompatible polymers that can easily be eliminated from the body and present no systemic toxicity. Polymerization techniques that allow more uniform polymers with a narrow dispersity should be considered to avoid variability in pharmacokinetic profiles, safety, and efficacy. The molar mass should be carefully chosen as large polymers may present more risk of toxicity with

reduced elimination rates. However, even low-molar mass polymers, usually considered non-toxic, should be evaluated carefully as they can still damage cellular membranes [166].

There are still many possibilities for developing GC-polymer conjugates as polymer therapeutics continue to be explored, and new polymers are tested as drug carriers to improve delivery. Biodegradable polymers such as poly(malic acid) have been developed as drug carriers in cancerous diseases [167], and new release strategies such as thermoresponsive polymers [168] have yet to be tested with GC.

6 Conflict of interest

The authors declare no conflict of interest.

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