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Glucocorticoid-loaded core-crosslinked polymeric micelles with tailorable release kinetics for targeted rheumatoid arthritis therapy**

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Abstract

To achieve highly effective glucocorticoid targeting for rheumatoid arthritis therapy, novel, polymerizable and hydrolytically cleavable dexamethasone (DEX) derivatives were covalently entrapped in core-crosslinked polymeric micelles. By varying the oxidation degree of the thioether in the drug linker, the release rate of DEX could be tightly controlled. Upon administration of the most rapidly releasing DEX-micelles, highly efficient disease treatment was achieved in two different animal models of inflammatory arthritis.

Keywords

Dexamethasone; Drug Delivery; Micelles; Nanomedicine; Rheumatoid Arthritis

Glucocorticoids (GC) play a prominent role in the therapeutic management of rheumatoid arthritis (RA) and other chronic inflammatory diseases.^[1] However, the occurrence of significant adverse effects, especially when high doses are required, forms an important limitation with respect to the clinical application of GC.^[1] Targeted delivery strategies for improving the target tissue accumulation of GC have therefore attracted considerable attention in recent years, especially for RA therapy.^[2] In RA, such strategies commonly exploit the up to 40-fold increase in blood-joint barrier permeability of the inflamed synovium for macromolecules and nanoparticulates.^[2b, 3] This preferred distribution of systemically administered nanomedicines to arthritic joints enables the inflammation-directed targeting of GC, thereby improving efficacy and reducing systemic GC toxicity.^[2a, 4]

In the last decades, many types of nanomedicines, including liposomes,^[5] polymeric drug-derivatives,^[6] protein-drug conjugates,^[7] polymeric micelles,^[8] and other types of nanoparticles,^[9] have been developed for drug targeting. Specific properties, such as size, circulation time, (bio)degradability, cellular distribution and internalization, and drug release kinetics, are important factors determining the efficacy of a system for a given therapeutic application. Core-crosslinked polymeric micelles (PM) based on poly(ethylene glycol)-*b*-poly(*N*-(2-hydroxypropyl)methacrylamide-lactate) (mPEG-*b*-pHPMAMLac_n) have attracted

considerable attention because of their favorable characteristics in this regard. Their (1) degradability under physiological conditions, (2) 50–100 nm size range (which is very suitable for drug targeting purposes), (3) prolonged circulation kinetics, and (4) flexibility with regard to their physicochemical properties, make core-crosslinked PM highly suitable for systemic drug targeting.^[10]

Following promising results obtained with PM for tumor-targeting of doxorubicin,^[11] we here for the first time demonstrate the potential of PM for GC targeting to joints affected by RA. Since the favorable circulation kinetics of core-crosslinked polymeric micelles in mice have been demonstrated previously,^[12] this study focuses on a novel chemical concept of using thioether esters to tailor the release of the targeted drug. To this end, dexamethasone (DEX), a highly potent GC, was derivatized with three different methacrylated linkers via ester bonds, thereby creating polymerizable DEX derivatives. By employing linkers that additionally contain thioethers with different degrees of oxidation, the hydrolysis rate of the ester bond, and therefore the drug release rate upon entrapment into the micellar core, is potentially controllable. The tailorable drug release kinetics of the different DEX-loaded PM were confirmed in vitro, and the anti-arthritic efficacy of the micellar nanomedicine with the most rapid release kinetics was evaluated in two different animal models of inflammatory arthritis.

PM were prepared from block copolymers consisting of a hydrophilic PEG block and a thermosensitive pHPMAmLac_n block, which is either hydrophilic or hydrophobic depending on the temperature (Figure 1A).^[13] At a temperature below its lower critical solution temperature (LCST), the pHPMAmLac_n block is hydrophilic, and therefore, in aqueous conditions, is fully soluble. However, when the temperature is raised above its LCST, the pHPMAmLac_n block will become hydrophobic, rendering the block copolymer amphiphilic, which in aqueous media results in the formation of micellar nanostructures composed of a hydrophobic pHPMAmLac_n core and a hydrophilic PEG shell. Subsequent crosslinking of the micellar core by polymerization of the partially methacrylated lactate side chains of the pHPMA backbone ensures particle stability below the critical micelle concentration (CMC), thereby preventing rapid dissociation upon systemic administration, and assuring prolonged circulation times.^[14]

Over time, the ester bonds linking the lactate groups and crosslinks to the polymeric backbone will undergo hydrolysis in aqueous conditions, which eventually results in destabilization of the crosslinked core. The outer shell of the PM is composed of hydrophilic PEG blocks, which is a key component for providing the PM with stealth-like properties, resulting in prolonged circulation times and efficient drug targeting to tumors and to sites of inflammation.^[12, 15]

To allow transiently stable entrapment of DEX in the PM, three different methacrylate-functionalized linkers were synthesized (Scheme 1). In a final single reaction step, these polymerizable linkers were coupled with high efficiency to dexamethasone, thereby generating polymerizable DEX derivatives. To prepare DEX-loaded PM, the hydrophobic DEX derivatives were first loaded into the micellar core during micelle formation, and subsequently covalently entrapped in the particle core upon free radical polymerization of the methacrylate moieties (Figure 1B). A highly efficient encapsulation of the DEX derivatives (>80%) was obtained at drug/polymer feed ratio of 10% (w/w). Consequently, the ratio of polymer methacrylate units versus the number of DEX derivatives was calculated to be 1.8. Importantly, it was shown that quantitative methacrylate conversion took place, as determined using a previously established HPLC method,^[16] indicating that all entrapped DEX derivatives were covalently incorporated in the polymeric structure. The

resulting DEX-loaded core-crosslinked PM were relatively monodisperse with a diameter of around 70 nm and a polydispersity index of 0.1.

To allow for tailorable release kinetics, hydroxyethyl-methacrylate was conjugated to dexamethasone via a sulfide (DMSL1), a sulfoxide (DMSL2), and a sulfone (DMSL3) ester. The presence of an electron-withdrawing moiety in close proximity of the ester bond reduces the electron density of the ester bond, which in turn accelerates the hydroxyl ion-driven hydrolysis of this bond, leading to a more rapid release of the conjugated drug.^[17] The linkers presented here contain a thioether, which possess higher electron-withdrawing properties as compared to an ether moiety.^[18] Moreover, the degree of oxidation of the sulfur in the thioether can be modified, which is expected to change its electron-withdrawing properties, allowing the adjustment of the hydrolysis rate of the neighboring ester bond. These linkers with different hydrolysis kinetics ensure proper release of DEX over time, and allow the rate of release from the core-crosslinked PM to be tailored. Indeed, under physiological conditions (37 °C, pH 7.4), when compared to the relatively slow DEX release from PM containing sulfide ester-linked DEX (DMSL1, < 5% in 7 days), the DEX release from PM containing the sulfoxide ester-linked DEX (DMSL2, $t_{1/2}$ 18.4 ± 0.4 d), or the sulfone ester-linked DEX (DMSL3, $t_{1/2}$ 8.9 ± 0.1 d) was much faster (Figure 2A). When the ester hydrolysis was accelerated by incubating the PM at pH 9.4, the DEX release, as expected, followed first-order kinetics where DMSL3 ($t_{1/2}$ 3.2 ± 0.3 h) was cleaved faster than DMSL2 ($t_{1/2}$ 7.1 ± 0.4 h), which in turn was cleaved considerably faster than DMSL1 ($t_{1/2}$ 41.3 ± 1.5 h) (Figure 2B). The release kinetics at pH 9.4 were approximately a factor 100 faster than at pH 7.4, demonstrating that the hydrolysis reaction was driven by OH⁻.^[19] These data convincingly demonstrate that the release kinetics of DEX from core-crosslinked PM can be tailored by employing DEX derivatives containing thioether esters with different degrees of oxidation, i.e. a sulfide, sulfoxide, or sulfone ester.

To demonstrate that PM hold significant potential as nanomedicines for systemic RA treatment, the anti-arthritis efficacy of DEX-PM prepared using DMSL3 (selected because of the highest DEX release rate, taking into account the acute inflammatory character of both animal models used) was evaluated in vivo. To this end, mice with collagen antibody-induced arthritis (CAIA; a model characterized by acute and strong joint inflammation)^[20] were treated intravenously (i.v.) with a single administration of PBS, unloaded PM, free DEX or DEX-PM, 5 days after disease induction. By using different doses of free DEX and DEX-PM, i.e. 1 mg/kg, 5 mg/kg and 10 mg/kg, the dose-dependent efficacy of DEX-PM in relation to that of the free drug was assessed.

As shown in Figure 3A, treatment with free DEX merely resulted in a moderate, dose-dependent and short-term reduction in clinical signs of arthritis, which was only significant on day 7, 8 and 10 for 5 mg/kg ($p < 0.05$, two-way ANOVA vs PBS), and on day 6 to 9 ($p < 0.05$) for 10 mg/kg (Figure 3A). In contrast, mice treated with 5 mg/kg or 10 mg/kg DEX-PM showed a strong and long-lasting reduction in arthritic symptoms as compared to controls ($p < 0.01$ from day 6 onwards, in case of 5 mg/kg; $p < 0.001$ from day 6 onwards, in case of 10 mg/kg; Figure 3B). Moreover, mice receiving DEX-PM dosed at 10 mg/kg showed no or very little signs of arthritis from day 9 onwards (i.e. 4 days after treatment), as illustrated by the arthritis scores that were comparable to those of healthy mice. Since PM have a circulation half-life of around 1 day,^[12] these long-lasting anti-inflammatory effects likely result from a preferential accumulation of the nanocarriers in inflamed joints, thereby enabling local release of dexamethasone, and not merely from an extension of the circulation kinetics of the GC. The pronounced improvement in therapeutic efficacy of DEX upon encapsulation in PM is further illustrated by the total disease load (defined as the area under the arthritis score curve) of DEX-PM-treated mice as compared to that of free DEX-

treated mice (Figure 3C, $p < 0.001$, one-way ANOVA). The change in ankle diameter, as a measure of inflammation-related joint swelling, confirmed these findings (Figure S1).

Subsequently, DEX-loaded PM were also evaluated in rats with adjuvant-induced arthritis (AA; a widely used arthritis model displaying a T lymphocyte-mediated inflammatory response against cartilage proteoglycan, which is in many respects similar to human RA).^[21] In this case, rats with established AA-induced polyarthritis were treated either with 4 daily i.p. injections of free Dex (2.5 mg/kg, total dose 10 mg/kg) or with a single i.v. administration of DEX-PM (Dex equivalent, 10 mg/kg), unloaded PM or PBS (Figure 4). Although the administration of free DEX resulted in a significant alleviation of the clinical signs of arthritis, as indicated by the arthritis score (Figure 4A; $p < 0.001$ as compared to PBS; from day 17 till 21; two-way ANOVA) and ankle swelling (Figure S2; $p < 0.001$; from day 17 until 23; $p < 0.05$ on day 25), this effect was only transient and started to weaken from day 5 after treatment. In contrast, upon treatment with DEX-PM at the same dose, an immediate and prolonged anti-arthritic effect was achieved ($p < 0.001$ as compared to PBS; from day 16 onwards; for both arthritis score and ankle diameter). DEX-PM was significantly more effective than free DEX in terms of reducing the arthritis score ($p < 0.05$ on day 16 and 20; $p < 0.001$ from day 21 onwards), as well as minimizing the degree of ankle swelling ($p < 0.001$ on day 23 and 25). Finally, while there was a disease load-limiting effect of 10 mg/kg of free DEX ($p < 0.01$ as compared PBS; one-way ANOVA), the total disease load of arthritic rats treated with DEX-PM was considerably lower than the disease load of rats treated with either PBS or free DEX ($p < 0.001$ for both) (Figure 4B).

An important advantage of the PM system for glucocorticoid targeting presented here is the tailorability of the release rate of dexamethasone from the carrier system, which allows further optimization of drug release kinetics for the treatment of RA, as well as other diseases. Future experiments will have to elucidate what are e.g. the optimal release kinetics for the treatment of different diseases. In addition, by incorporating a combination of DEX derivatives with different conversion rates within a single micellar nanomedicine, it will be possible to fine-tune the DEX release profile, e.g. by employing a rapid-release and with a slow-release component. Another advantage of the presented system is the entrapment of the therapeutic agent in the hydrophobic core of the PM, which limits any unwanted exposure to its environment. Finally, since the synthesis procedure for preparing polymerizable DEX-derivatives only requires the drug to be present in the final step of the process, the linker technology presented here is broadly applicable for preparing core-crosslinked PM or other (polymeric) nanocarriers entrapping a wide range of therapeutic agents, i.e. compounds containing a reactive hydroxyl moiety, making this system very versatile and promising, not only for targeted RA therapy but for many other disorders.

Experimental Section

Full experimental details are available on the WWW under <http://www.angewandte.org>

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic representation of dexamethasone-loaded core-crosslinked polymeric micelles (DEX-PM). **a)** Chemical structure of thermosensitive poly(ethylene glycol)-*b*-poly(*N*-(2hydroxypropyl)-methacrylamide-lactate) (mPEG-*b*-pHPMAMLac_n) block copolymers. **b)** Preparation, degradation, and drug release from DEX-PM. (1) Micelle formation and encapsulation of hydrophobic DEX derivatives by rapid heating; (2) copolymerization of methacrylated lactate side chains and DEX derivatives in the micelle core; (3) hydrolytic liberation of lactate moieties, drugs and drug linkers, leading to micellar destabilization and drug release.

Figure 2.

The release of DEX from core-crosslinked PM loaded with different DEX derivatives at 37°C and pH 7.4 (a) or pH 9.4 (b). DEX is covalently linked to the hydrophobic core of the micelle via hydrolysable linkers based on a sulfide ester (●, DMSL1), a sulfoxide ester (■, DMSL2) or a sulfone ester (▲, DMSL3) (Scheme 1). The release rate from the PM was dependent on the degree of oxidation of the sulfur atom in the DEX-linker, with the order in release rate: DMSL3 > DMSL2 > DMSL1.

Figure 3.

Therapeutic efficacy of free dexamethasone phosphate (DEX) and dexamethasone-loaded polymeric micelles (DEX-PM) in mice with collagen antibody-induced arthritis (CAIA). Polyarthritis was induced by i.v. injection of a collagen antibody cocktail on day 0, followed by an i.p. administration of LPS on day 3. **a)** and **b)** The arthritis score, representing disease activity, was determined daily using the scoring system presented in Table S1. On day 5 (arrow), when all CAIA-induced mice presented with clear signs of inflammation, they received an i.v. injection of DEX (A) or DEX-PM (B) dosed at 1 mg/kg (■), 5 mg/kg (▲), or 10 mg/kg (▼). Control mice (●) received PBS or unloaded micelles. One group of mice without CAIA served as healthy control (◇). The data is presented as the mean score with SEM of each group. **c)** The disease load of each individual mouse upon treatment with free DEX (○) or DEX-PM (■). The disease load was defined as the area under the arthritis score curve from treatment (day 5) until the end of the study (day 13). The mean of the experimental group is presented as a straight line (dashed for free DEX; solid for DEX-PM). Control animals were injected with PBS (free DEX group) or unloaded micelles (DEX-PM group). At a dose of 5 mg/kg or 10 mg/kg, but not at 1 mg/kg, there was a significant reduction of mean disease load after treatment DEX-PM when compared to free DEX (one-

way ANOVA followed by Bonferroni's multiple comparison test). ***, $p < 0.001$; ns, not significant.

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Figure 4.

Therapeutic efficacy of free dexamethasone phosphate (DEX) and dexamethasone-loaded polymeric micelles (DEX-PM) in rats with adjuvant-induced arthritis (AA). Arthritis was induced on day 0 by subcutaneous administration of *Mycobacterium tuberculosis* H37Ra in the base of the tail. On day 15 (arrow), rats ($n=7$, all groups) with established AA were treated with 4 daily i.p. injections of free Dex (2.5 mg/kg, total dose 10 mg/kg, ■) or a single administration of DEX-PM (Dex equivalent 10 mg/kg, △). Control rats received PBS (●), and one group of rats without AA served as healthy controls. **a)** The arthritis score, representing disease activity, of all rats (shown as mean with SEM of each group), determined daily using the scoring system presented in Table S2. **b)** The disease load, defined as the area under the arthritis score curve from treatment (day 15) until the end of the study (day 25), of each individual rat, presented together with the mean of the treatment group as a straight line. The disease load of animals in each treatment group was significantly different from those in the other groups ($p<0.001$, one-way ANOVA followed by Bonferroni's multiple comparison test).

Scheme 1.

Synthesis of polymerizable sulfide (DMSL1), sulfoxide (DMSL2), and sulfone (DMSL3) ester dexamethasone derivatives.

a) Synthesis of methacrylated linkers containing a thioether moiety with different degrees of oxidation a. 2-mercaptoethanol, Et₃N, CH₂Cl₂, b. methacryloylchloride, Et₃N, CH₂Cl₂, c. trifluoroacetic acid, d. NaIO₄, MeCN/ H₂O, e. NaIO₄, RuCl₃, MeCN/ CCl₄/ H₂O

b) Conjugation of the linkers to dexamethasone in a single reaction step to create polymerizable, hydrolytically cleavable dexamethasone derivatives.