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HPMA-based polymer conjugates with drug combination

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ABSTRACT

Synthesis and physico-chemical behavior of new polymer–drug conjugates intended for the treatment of cancer were investigated. In the polymer conjugate with the expected dual therapeutic activity, two drugs, a cytostatic agent doxorubicin (DOX) and anti-inflammatory drug dexamethason (DEX) were covalently attached to the same polymer backbone via hydrolytically labile pH-sensitive hydrazone bonds. The precursor, a copolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) bearing hydrazide groups randomly distributed along the polymer chain, was conjugated with DOX (through its C13 keto group) or with a keto ester (DEX). Two derivatives of DEX, 4-oxopentanoate and 4-(2-oxopropyl)benzoate esters, were synthesized and employed for conjugation reaction. As a control, also a few polymer conjugates containing only a single drug (DOX or DEX) attached to the polymer carrier were synthesized. Physico-chemical properties of the polymer conjugates strongly depend on the attached drug, spacer structure and the drug content. Polymer–drug conjugates incubated in buffers modeling intracellular environment released the drug (DOX) or a drug derivatives (DEX) at the rate significantly exceeding the release rate observed under conditions mimicking situation in the blood stream. Incubation of the DEX conjugates in a buffer containing carboxyesterase resulted in complete ester hydrolysis thus demonstrating susceptibility of the system to release free active drug in the two-step release profile.

1. Introduction

The anti-cancer drugs mostly consist of low-molecular-weight compounds hence they are very quickly excreted from organism by glomerular filtration or entrapped by the reticulo-endothelial system and metabolized (Ringsdorf, 1975). The main disadvantages of such anti-cancer low-molecular-weight drugs are non-specific body distribution, low bioavailability and various, often toxic side effects. With the aim to reduce the side effects, improve the drug distribution in the body, prolong its blood circulation and persistence in the body, the low-molecular-weight drugs were incorporated into polymeric nanoparticles and liposomes or covalently conjugated to water-soluble polymer carriers. Previously, many types of polymers were used as water-soluble drug carriers, e.g. poly(L-glutamic acid) (Li, 2002; Tansey et al., 2004), poly(ethylene glycol) (Veronese and Pasut, 2005) and its biodegradable multiblock polymers (Pechar et al., 2001, 2005), *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers (Etrych et al., 2001; Kopeček and Duncan, 1987; Ulbrich et al., 2003) or poly(vinylpyrrolidone) (Bharali et al., 2003; Yamamoto et al., 2004). HPMA copolymers rank among the most intensively studied polymer–drug carrier systems. Some of the HPMA copolymer–drug conjugates have been subjected to clinical trials (Julyan et al., 1999; Vasey et al., 1999). Previously, the anti-cancer drug doxorubicin (DOX) was attached to the HPMA copolymers via enzymatically degradable oligopeptide spacer GlyPheLeuGly (GFLG) (Duncan et al., 1983; Ulbrich et al., 1980) or via a hydrolytically labile spacer containing hydrazone bond susceptible to pH-controlled hydrolysis (Etrych et al., 2001, 2002). The oligopeptide spacer GFLG used in the HPMA copolymer–DOX conjugate was tailored for specific enzymatic cleavage by lysosomal enzymes in lysosomes of the target cells, the spacer was stable during blood circulation. The conjugates containing spacer with the pH-sensitive hydrazone linkage were fairly stable in model buffers at pH 7.4, simulating blood pH, and released the drug by chemical hydrolysis at pH modeling the endosomal and lysosomal environment inside the target cell (pH 5–6).

It was shown previously that high-molecular-weight polymers (above the limit of the renal threshold) accumulate in tumor tissues at much higher concentrations than in normal tissues or organs. Maeda defined such accumulation of polymers in solid tumors as the enhanced permeability and retention effect (EPR effect) (Maeda and Matsumura, 1989). The EPR effect is caused by the 'leaky' endothelium of angiogenic tumor vasculature and lack of effective tumor lymphatic drainage. Therefore, the polymers in solid tumors can easily extravasate but cannot be removed by the lymphatic sys-

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tem and remain entrapped in the tumor (Matsumura and Maeda, 1986).

Recently, in agreement with new findings in oncology, a new approach in designing polymer–drug conjugates intended for treatment of cancer has appeared—the polymer–drug conjugates bearing a combination of two drugs differing in their mechanism of action, both attached to the same carrier via biodegradable spacers (Kopeček et al., 1990; Shiah et al., 2001; Vicent et al., 2005). HPMA copolymer conjugates, with combination of DOX and BS-RNase or DOX and RNase A exhibited a synergistic effect in vivo and effectively inhibited growth of melanoma, solid tumors and metastases in mice (Poučková et al., 2004; Souček et al., 2001, 2002).

Here we describe the synthesis and physico-chemical characteristics of the new types of conjugates using HPMA copolymers as drug carriers, bearing the anti-inflammatory and anti-cancer drug dexamethasone or a combination of two anti-cancer drugs, DOX and DEX. DEX has been used for treatment of many inflammatory and autoimmune diseases, malignant diseases such as lymphatic leukemia, lymphomas and multiple myeloma. DEX has been also used for treatment of cancer patients undergoing chemotherapy to eliminate side effects of the treatment. It was shown that treatment of multiple myeloma and other types of cancer with DEX combined with anti-cancer drugs, e.g. doxorubicine, vincristine, bortezomid or thalidomide (Alexanian et al., 1992; Hussein et al., 2002; Oakervee et al., 2005; Tosi et al., 2004) represents an effective method that produces more rapid response than other regimens (Hussein, 2003). Unfortunately, long-lasting continuous infusion is required for such treatment. Use of a polymer carrier bearing both drugs and releasing them simultaneously for long period of time can help to overcome this problem and bring improvement into the treatment.

Various spacers enabling pH-dependent hydrolytically controlled drug release were described as suitable structures for designing polymer–drug conjugates with anti-cancer activity (Ulbrich and Šubr, 2004). In the conjugates used in this study the drug or its derivative was attached to the polymer carrier via hydrolytically degradable hydrazone bond. In the DEX-containing conjugates the drug was first esterified with 4-oxopentanoic acid (levulic acid, LEV) or 4-(2-oxopropyl)benzoic acid (OPB) and then the respective ester derivate was attached to the polymer via hydrazone bond in the same way as in the case of DOX.

Several polymer conjugates containing either single drug (DOX, DEX derivative), or combination of both drugs were synthesized and their physico-chemical properties were studied. The rates of release of the drugs or their derivatives from the polymer conjugates were studied *in vitro* in buffers modeling blood circulation or intracellular environment.

2. Experimental

2.1. Chemicals

N,*N*′-dicyclohexylcarbodiimide (DCC), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), dimethylformamide (DMF), 4-(dimethylamino)pyridine (DMAP), dichloromethane, methanol, tetrahydrofuran, 2,2′-azobis(isobutyronitrile) (AIBN), 1-aminopropan-2-ol, methacryloyl chloride, methyl 6-aminohexanoate hydrochloride (ah), hydrazine hydrate, dexamethasone (DEX), doxorubicin hydrochloride (DOX·HCl) and 4-oxopentanoic acid were purchased from Fluka. 4-(2-Oxopropyl)benzoic acid was obtained from Rieke Metals. 2,4,6-Trinitrobenzene-1-sulfonic acid (TNBSA) was purchased from Serva, Heidelberg. Germany. Rabbit liver carboxyesterase (EC 3.1.1.1) was obtained from Sigma–Aldrich.

2.2. Synthesis of monomers

N-(2-Hydroxypropyl)methacrylamide (HPMA) was synthesized by methacryloylation of 1-aminopropan-2-ol as described earlier (Ulbrich et al., 2000) using Na_2CO_3 as a base. M.p. 64–66 °C; elemental analysis: calculated C 58.74, H 9.0, N 9.79; found: C 58.81, H 9.09, N 9.82.

6-Methacrylamidohexanohydrazide (Ma-ah-$NHNH_2$) was prepared as described in the literature (Etrych et al., 2008). M.p. 79–81 °C; elemental analysis: calculated C 56.32, H 8.98, N 19.70; found: C 56.49, H 8.63, N 19.83.

Purity of monomers was examined in Shimadzu HPLC system equipped with a reverse-phase column Chromolith Performance RP-18e (100 mm × 4.6 mm) (water–acetonitrile, gradient 0–100%

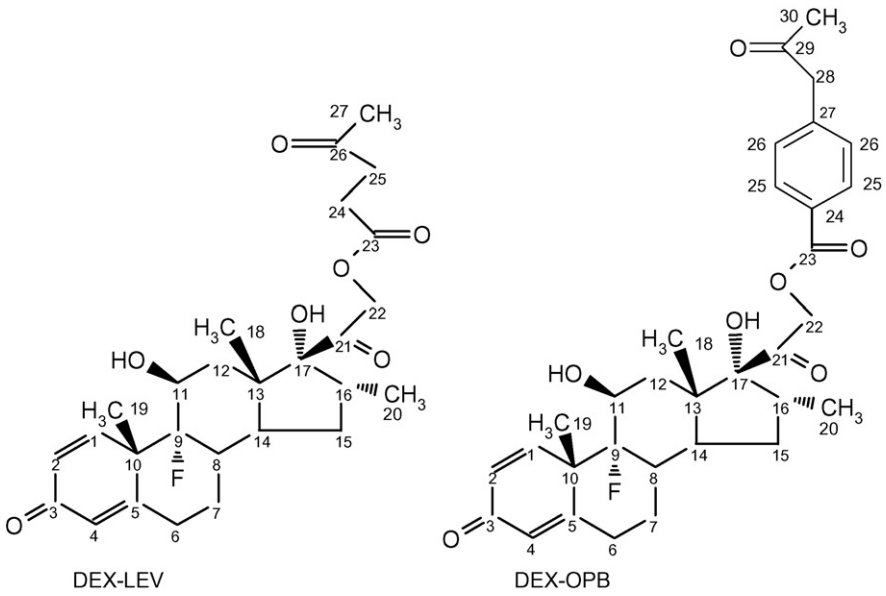

Fig. 1. Dexamethasone derivatives.