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Tumor-targeted micelle-forming block copolymers for overcoming of multidrug resistance

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ABSTRACT

New amphiphilic diblock polymer nanotherapeutics serving simultaneously as a drug delivery system and an inhibitor of multidrug resistance were designed, synthesized, and evaluated for their physico-chemical and biological characteristics. The amphiphilic character of the diblock polymer, containing a hydrophilic block based on the *N*-(2-hydroxypropyl)methacrylamide copolymer and a hydrophobic poly(propylene oxide) block (PPO), caused self-assembly into polymer micelles with an increased hydrodynamic radius (R_h of approximately 15 nm) in aqueous solutions. Doxorubicin (Dox), as a cytostatic drug, was bound to the diblock polymer through a pH-sensitive hydrazone bond, enabling prolonged circulation in blood, the delivery of Dox into a solid tumor and the subsequent stimuli-sensitive controlled release within the tumor mass and tumor cells at a decreased pH. The applicability of micellar nanotherapeutics as drug carriers was confirmed by an *in vivo* evaluation using EL4 lymphoma-bearing C57BL/6 mice. We observed significantly higher accumulation of micellar conjugates in a solid tumor because of the EPR effect compared with similar polymer-drug conjugates that do not form micellar structures or with the parent free drug. In addition, highly increased anti-tumor efficacy of the micellar polymer nanotherapeutics, even at a sub-optimal dose, was observed. The presence of PPO in the structure of the diblock polymer ensured, during *in vitro* tests on human and mouse drug-sensitive and resistant cancer cell lines, the inhibition of P-glycoprotein, one of the most frequently expressed ATP-dependent efflux pump that causes multidrug resistance. In addition, we observed highly increased rate of the uptake of the diblock polymer nanotherapeutics within the cells. We suppose that combination of unique properties based on MDR inhibition, stimuli sensitiveness (pH sensitive activation of drug), improved pharmacokinetics and increased uptake into the cells made the described polymer micelle a good candidate for investigation as potential drug delivery system.

1. Introduction

Multidrug resistance (MDR) is one of the main causes of the imperfect or even ineffective treatment of many types of haematological malignancies and solid tumors. The resistance of cancer cells to current chemotherapy is associated with the overexpression of at least two ATP-dependent efflux pumps, P-glycoprotein (P-gp; MDR1) and multidrug resistance-associated protein 1 (MRP1), both belonging to a superfamily of ATP-binding cassette (ABC) transporters [1,2]. These overexpressed proteins participate in the preferential active transport of cytotoxic drugs out of cancer cells before the therapeutic effect of drugs. Thus, the intracellular concentration of drugs in tumors is decreased, and conversely, the probability of the survival of tumor cells increases. An inhibition of the membrane transporters [3–5] should improve the retention of chemotherapeutics inside tumor cells, allowing a more effective cancer therapy to be achieved. The application of low-molecular weight (LMW) P-gp inhibitors (cyclosporine A, verapamil, ritonavir, vincristine, tamoxifen, *etc.*) with non-selective biodistribution represents a serious risk associated with the inhibition of P-gp in healthy tissues. Although P-gp protects the organism against xenobiotics in normal cells, this protein contributes to the resistance of tumors towards cytostatic drugs in cancer cells.

It has been previously found [6–11] that the high-molecular weight (HMW) polymer carriers and their polymer-drug conjugates are subject to so-called passive accumulation in solid tumors. This phenomenon is called the EPR (enhanced permeability and retention) effect [6,12–15]. HMW compounds are preferably accumulated in solid tumors because of the leaky tumor neovasculature, but they are not eliminated because of the damaged or completely missing lymphatic drainage in solid tumor tissues. Currently, the EPR effect is highly utilized in the design and application of polymer-drug conjugates tailor-made for passive accumulation in solid tumors. The use of HMW polymer-drug conjugates also offers an option for the inhibition of P-gp, provided that part of the

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polymer carrier acts as a P-gp inhibitor. Kabanov and coworkers [2,16–21] studied micellar carriers based on various types of pluronic triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), where PPO block was responsible for sensitizing resistant cells to cytostatics. Consequently, the cytotoxic activity of the drug was increased [2]. The major and limiting disadvantage of these systems with non-covalently incorporated drugs in the hydrophobic core of the micelle could be continuous non-controlled drug release within the whole organism caused by diffusion and micelle disintegration during its circulation in blood. Recently, micellar polymer systems become extensively studied as drug delivery systems. Various amphiphilic block copolymer, including amphiphilic block copolymer with poly(*N*-(2-hydroxypropyl)methacrylamide) synthesized using RAFT polymerization as the hydrophilic block, were studied as precursors for self-assembling micellar systems [22–24].

Here, we present the design, synthesis and evaluation of the physico-chemical and biological properties of amphiphilic diblock copolymers containing a hydrophilic copolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) with 1-(*tert*-butoxycarbonyl)-2-(6-methacrylamidohexanoyl)hydrazine (Ma-Ah-NHNH-Boc) and hydrophobic PPO. We hypothesized that the replacement of the hydrophilic PEO block in the pluronic polymer with another hydrophilic polymer, such as a HPMA copolymer, might lead to block copolymers with similar P-gp-inhibiting properties as the pluronic polymer. At the same time, the block copolymer may serve as a carrier of an anticancer drugs, like doxorubicin (Dox), covalently linked to the copolymer *via* a pH-sensitive hydrazone bond, which is biodegradable inside cancer cells and stable during the transport in the organism [2,15,25–28]. The amphiphilic dual character of the synthesized diblock polymer precursor **DB** (Synthesis on Fig. 1A) and the polymer-drug conjugate **DB-Dox** (Fig. 1B) enables the formation of micelles in aqueous environments (Fig. 1C) as well as increases in apparent molecular weight and size in solution. This provides a great advantage for the preferential passive targeting of the system into solid tumors driven by the EPR effect. After the disintegration of the micelles to unimers because of their gradual dilution below the critical micellar concentration (CMC) in targeted cells/tumor tissue, the unimers should be excreted from the organism by glomerular filtration, as their molecular weight is well below the renal filtration limit (M_n = 12,950 g/mol; Đ = 1.18). We found that this micellar system can serve as both a passively targeted drug delivery system and a polymer inhibitor of P-gp because of the presence of the PPO block in its structure. In consequence, the proposed polymer system could be a good candidate for the effective treatment of various tumors, even those that are resistant.

2. Material and methods

2.1. Synthesis of the monomers and initiator

N-(2-Hydroxypropyl)methacrylamide (HPMA) was synthesized according to the literature [29]. M.p. 69–70 °C; Elemental analysis: calculated/found: C 58.72/58.98%, H 9.15/9.18%, N 9.78/9.82%. 1-(*tert*-Butoxycarbonyl)-2-(6-methacrylamidohexanoyl)hydrazine (Ma-Ah-NHNH-Boc) was prepared as described previously [30]. M.p. 114–116 °C; Elemental analysis: calculated/found: C 57.49/58.26%; H 8.68/8.95%; N 13.41/13.25%.

2-[1-Cyano-1-methyl-4-oxo-4-(2-thioxo-thiazolidin-3-yl)-butylazo]-2-methyl-5-oxo-5-(2-thioxothiazolidin-3-yl)-pentanenitrile (ABIC-TT) was prepared as described previously [31]. Elemental analysis:

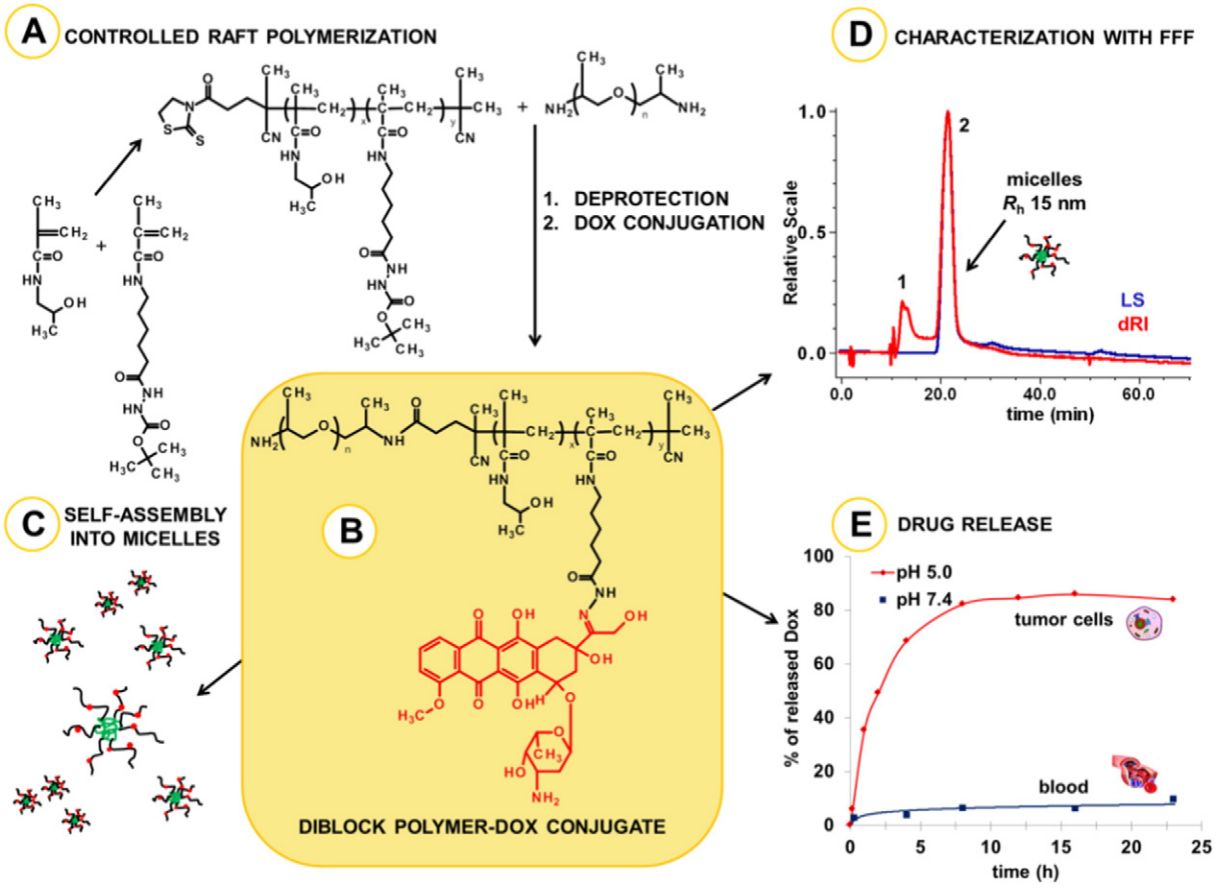

Fig. 1. Overall description of the micellar diblock polymer system with Dox: synthesis (A), schematic structure (B), self-assembly into micellar structures (C), characterization with the FFF method (D), and drug release in aqueous media mimicking the conditions of the bloodstream and intracellular environment (E).