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Dual-pH responsive micelle platform for co-delivery of axitinib and doxorubicin

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ABSTRACT

While the complicated pathogenesis of cancer results in limited therapeutic efficacy of current mono-drug treatment, combination therapy by multiple drugs is becoming increasingly attractive due to the decreased side effects and synergistic anti-cancer activities. The recently emerging modality is the co-delivery of traditional chemotherapeutics and anti-angiogenesis agents. Although nanocarriers are frequently utilized for the co-delivery of different drugs, there are still concerns regarding their implementations. Most of the nanocarriers cannot release drugs separately into their different targeted sites of action. Therefore, we have developed a micellar platform for the co-delivery of an antiangiogenesis agent, axitinib (AXI) and a DNA intercalator, doxorubicin (DOX). Our results showed that this cross-linked micelle (DA-CM) could release AXI and DOX in tumor extracellular environment and intracellular lysosome compartments, respectively, in response to the dual pH stimulus. Notably, DA-CM exhibited remarkably improved tumor accumulation, cell internalization, tumor spheroids penetration and cytotoxicity. Ultimately, DA-CM reduced the number of immature vessels within xenograft tumors, demonstrating an effective antiangiogenesis effect. Meanwhile, they inhibited tumor growth by 88%. Our co-delivery micellar system with the dual-pH responsive feature might hold great promises for the combinatory cancer therapy.

1. Introduction

Recent advances in cancer nanomedicine are largely focused on drug delivery systems to improve the *in vivo* stability and pharmacokinetics of conventional drugs (Cao et al., 2014; Deng et al., 2012; Zamboni, 2005; Zhu et al., 2010). However, abnormal tumor vasculature greatly hinders the treatment efficacy, and partly contributes to the drug resistance and tumor progression (Guo et al., 2014; Jain and Stylianopoulos, 2010). Therefore, successfully remodeling the tumor vasculature may be a potent approach to sensitize tumor cells to chemotherapy.

Combinatory therapy has long been known to improve the tumor inhibition and decrease side effects (Desale et al., 2015; Greco and Vicent, 2009; Zhu et al., 2010). The combination of anti-vascular endothelial growth factor (VEGF) monoclonal antibodies and cytotoxic therapeutic agents is a clinically well-established modality for cancer treatment (Ellis and Hicklin, 2008; Gasparini et al., 2005; Hurwitz et al., 2004; Margolin et al., 2001; Romond et al., 2005). Synergy may be achieved as a result of increased cancer cell death after tumor vascular normalization, because increased blood flow in tumor sites can facilitate the nano-vehicles to penetrate faster and deeper through the tumor interstitial matrix (Jain et al., 2006; Tong et al., 2004). However, the non-specificity of anti-VEGF agents and free cytotoxic drugs can lead to serious systemic side effects including hypertension, life-threatening hemorrhage, and uncommon thrombotic events (Midgley and Kerr, 2005; Mross et al., 2005; Thomas et al., 2005). Therefore, it is urgent to establish an efficient carrier system to co-deliver anti-VEGF agents and traditional chemotherapeutic drugs for tumor-targeted therapy.

Several carrier systems have been exploited for the co-delivery of anti-VEGF and cytotoxic agents, such as microspheres, core-shell nanoparticles, and micelles (Carrasquillo et al., 2003; Torchilin, 2005; Zhang et al., 2015). Among them, micelles are one of the most effective systems due to the simplicity in manipulation, high efficiency in loading hydrophobic drugs, and suitable particle size that permits specific accumulation in solid tumor (Kataoka et al., 2001; Kedar et al., 2010). However, these co-delivery nanocarriers

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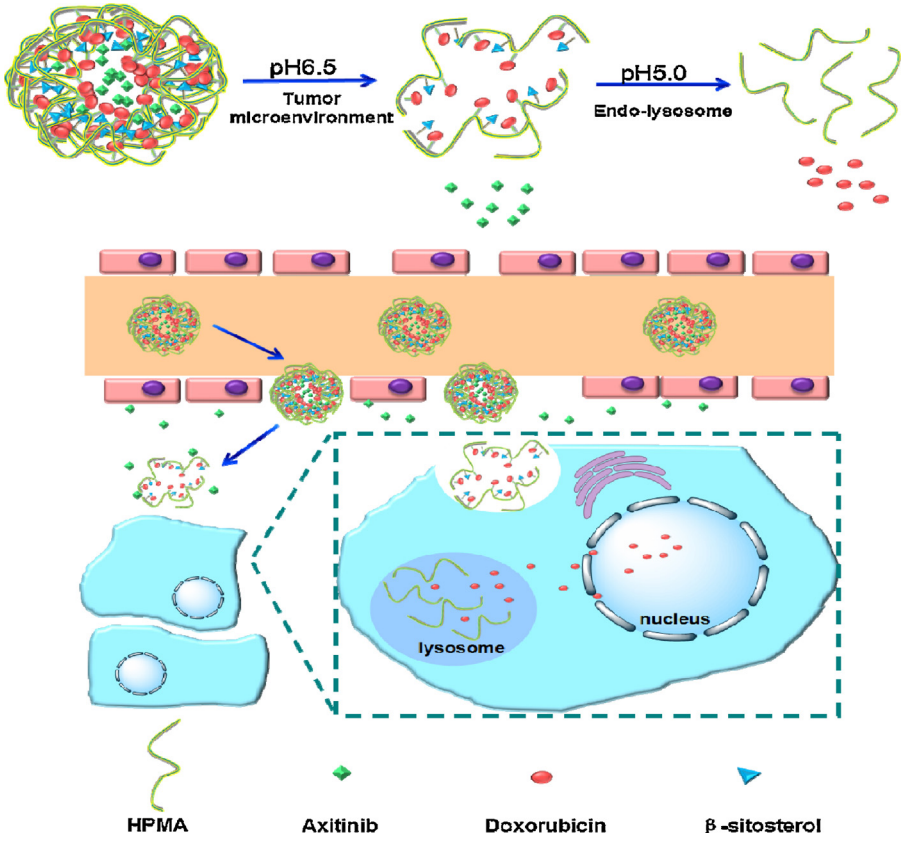
cannot response to abnormal tumor microenvironment and only release the encapsulated anti-VEGF agents inside the tumor cell. In this way, the interaction between anti-VEGF agents and their large amount of tyrosine kinase receptor on the cell surface could be bypassed and blocked.

Therefore, we have developed a dual-pH responsive cross-linked micelle for two-step release of a small molecule receptor tyrosine kinase inhibitor, axitinib (AXI) and a cytotoxic agent, doxorubicin (DOX). DOX was conjugated to amphiphilic N-(2-hydroxypropyl) methacrylamide (HPMA) polymers via pH sensitive hydrazone linkage (Li et al., 2015; Zhou et al., 2014b) for the selective transportation to tumor site due to the enhanced permeability and retention (EPR) effect (Maeda, 2015). Then AXI was further encapsulated into the self-assembly micelles for antiangiogenesis. Finally, benzoic-imine bonds (Quan et al., 2010; Xiao et al., 2014; Xu et al., 2011) were used to cross-link the micelles to improve the circulatory stability and induce tumor microenvironment responsiveness. We expected that the cross-linked micelle would escort AXI and DOX selectively to tumor site. Then, the tumor extracellular pH (pH 6.5) (Lee et al., 2008) would break down the benzoic-imine bond, which would allow for the release of AXI into the tumor microenvironment and ensure the AXI interaction with tyrosine kinase receptor located on the cell membrane for vasculature modulation. Finally, the second-stage pH responsiveness would occur after endocytosis, due to the more acidity-triggered hydrolysis of hydrazone linkages in lysosome (pH 5.0), and subsequently release the conjugated DOX to trigger cell death (Scheme 1).

2. Experimental

2.1. Materials

Doxorubicin hydrochloride (DOX·HCl) and axitinib (AXI) were purchased from Dalian Meilun Biotech Co., Ltd. (Dalian, China). β-sitosterol (SITO) was purchased from Chengdu Pushi Biotechnology Co., Ltd. (Chengdu, China). *N*-3-aminopropylmethacrylamidehydrochloride (APMA) was purchased from PolySciences. A series of functional monomers including *N*-(2-hydroxypropyl) methacrylamide (HPMA), *N*-methacryloylglycylglycyl-hydrazide-doxorubicin (MA-GG-NHN = DOX), *N*-methacryloylglycylglycyl-sitosterol ester (MA-GG-SITO), Cy-5 methacrylamide monomer (MA-Cy5) were synthesized in our laboratory as described. 3-(4,5-dimethyl-2-tetrazolyl)-2,5-diphenyl-2H tetrazoliumbromide (MTT), 2,4,6- trinitrobenzene-1-sulfonic acid (TNBSA), glycylglycine (GG), and 4′,6-diamidino- 2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*,*N*′-diisopropylcarbodiimide (DIC) was purchased from AstaTech (Chengdu, China). Cyanine 5 NHS ester (Cy5-NHS) was purchased from Lumiprobe (USA). Recombinant human VEGF165 (VEGF) was gained from Genentech. Goat anti-mouse fluorescein-conjugated immunoglobulin G was obtained from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China), and rat anti-CD31 antibody was purchased from BD Bioscience (San Diego, USA). All the other chemicals were of analytical grade.



Scheme 1. Illustration of dual-pH responsive micellar platform for co-delivery of axitinib (AXI) and doxorubicin (DOX) based on HPMA coplymers.