HEALTH AND MEDICINE

A combinational chemo-immune therapy using an enzyme-sensitive nanoplatform for dual-drug delivery to specific sites by cascade targeting

Yanmei He[1]*, Lei Lei[1,2]*, Jun Cao[1,3†], Xiaotong Yang[2], Shengsheng Cai[1], Fan Tong[2], Dennis Huang[3], Heng Mei[1], Kui Luo[4], Huile Gao[2†], Bin He[1], Nicholas A. Peppas[3,5†]

Nanoparticle-based drug delivery faces challenges from the imprecise targeted delivery and the low bioavailability of drugs due to complex biological barriers. Here, we designed cascade-targeting, dual drug–loaded, core-shell nanoparticles (DLTPT) consisting of CD44-targeting hyaluronic acid shells decorated with doxorubicin (HA-DOX) and mitochondria-targeting triphenylphosphonium derivative nanoparticle cores loaded with lonidamine (LND) dimers (LTPT). DLTPT displayed prolonged blood circulation time and efficiently accumulated at the tumor site due to the tumor-homing effect and negatively charged hyaluronic acid. Subsequently, the HA-DOX shell was degraded by extracellular hyaluronidase, resulting in decreased particle size and negative-to-positive charge reversal, which would increase tumor penetration and internalization. The degradation of HA-DOX further accelerated the release of DOX and exposed the positively charged LTPT core for rapid endosomal escape and mitochondria-targeted delivery of LND. Notably, when DLTPT was used in combination with anti–PD-L1, the tumor growth was inhibited, which induced immune response against tumor metastasis.

INTRODUCTION

In recent years, various nanoparticle-based drug delivery systems have been developed and widely used in cancer chemotherapy (*1*–*4*). Although nanoscale drug delivery systems can improve the bioavailability and tolerance of drugs, the efficacy of drug delivery is still restricted by the "CAPIR cascade," e.g., blood circulation, tumor accumulation, tumor penetration, tumor internalization of nanoparticles, and intracellular specific drug release (*5*–*7*). To overcome the obstacles associating with the CAPIR cascade, we developed cascade-targeting, dual drug–loaded, core-shell intelligent nanoparticles (DLTPT) with tumor-active targeting, enzyme-sensitive size modifications, charge reversal, and organelle-specific controlled-release properties. Hyaluronic acid (HA) was selected as the surface layer of DLTPT to facilitate the targeting of particles to CD44 receptor and the degradation by hyaluronidase (HAase), which are both overexpressed in the tumors (*8*, *9*). After degradation, the inner positively charged triphenylphosphonium derivatives particles (LTPT) with smaller size and negative-to-positive charge reversal were released to penetrate deep into the tumor, efficiently uptaken by tumor cells, followed by rapid endosomal escape. TPT was constructed by the self-assembly of the amphiphilic TPT polymer containing relatively hydrophilic triphenylphosphine (TPP) and biodegradable hydrophobic poly(L-lactic acid) (PLLA), in which the TPP could target the mitochondria for organelle-specific drug delivery and the PLLA could form hydrophobic cores to load drugs (*10*, *11*).

The combination of various intracellular-functioning small-molecule drugs has recently evolved as a burgeoning therapeutic strategy because it could lead to the destruction of specific subcellular structures via activating apoptotic signals (*12*, *13*). For instance, lonidamine (LND) interferes with mitochondrial function to activate apoptotic signaling by releasing cytochrome C into the cytoplasm and activating caspase-9, leading to the up-regulation of downstream caspase-3 (*14*, *15*). Similarly, doxorubicin (DOX) induces apoptosis via nuclear oxidative DNA damage, which could cause indirect hydrogen peroxide (H_2O_2) generation through poly ADP (adenosine diphosphate)–ribose polymerase (PARP) and triphosphopyridine nucleotide (NADPH) oxidase activation, leading to an increase in mitochondrial membrane potential and subsequent caspase-3 activation (*16*). Therefore, DOX and LND might show a synergistic antitumor effect. However, small-molecule drugs show no organ selectivity, thus typically failing to accumulate in specific organelles (*17*). To specifically deliver DOX and LND into different organelles, DOX was conjugated onto the HA chains to form the shell of DLTPT, which would release DOX into the cytoplasm, followed by its targeting to the nucleus, and LND was encapsulated within the TPT core via π-π stacking and electrostatic interactions, which would specifically deliver LND to the mitochondria. However, the weak interaction between small-molecule drugs and amphiphilic copolymers results in the low drug loading capacity (DLC) of LND (*18*, *19*). Therefore, LND dimer (d-LND) was synthesized using the sulfur bond as the linker to improve the DLC, and d-LND could intelligently produce LND in response to intracellular glutathione (GSH) to achieve specific release (*20*–*22*).

Chemotherapeutic agents, such as anthracyclines, are known to induce immunogenic cell death (ICD) (*23*–*25*). ICD can induce an immune response through the activation of dendritic cells (DCs) and the consequent activation of a T cell–specific response (*26*–*28*). However, the antitumor effect of tumor-reactive cytotoxic T cells was limited because of the overexpression of programmed cell death 1 ligand 1 (PD-L1) on cancer cells, which interacts with programmed cell death receptor 1 (PD-1) on T cells and induces T cell apoptosis (*29*–*31*). PD-L1 antibodies (anti–PD-L1) can block the immune

[1]National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, P. R. China. [2]West China School of Pharmacy, Sichuan University, Chengdu 610041, P. R. China. [3]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX 78731, USA. [4]Huaxi MR Research Center (HMRRC), Functional and Molecular Imaging Key Laboratory of Sichuan Province, Department of Radiology, West China Hospital, Sichuan University, Chengdu 610041, P. R. China. [5]Division of Molecular Pharmaceutics and Drug Delivery, College of Pharmacy, The University of Texas at Austin, Austin, TX 78731, USA.
*These authors equally contributed to this work.
†Corresponding author. Email: caojun@scu.edu.cn (J.C.); gaohuile@scu.edu.cn (H.G.); peppas@che.utexas.edu (N.A.P.)