An intelligent re-shieldable targeting system for enhanced tumor accumulation

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**A B S T R A C T**

Programmed ligand targeting strategy promotes the blood circulation stability of nanoparticles by shielding the ligand. However, the irreversible shielding causes the deshielded nanoparticles to be easily recognized and cleared by the reticuloendothelial system (RES), impeding their further retention in the tumor. Here, we for the first time prove the superiority of the intelligent re-shieldable targeting system that is based on the pH-responsive self-assembly/disassembly of gold nanoparticles. The system can enhance the stability of gold nanoparticles in the blood circulation (2.6-fold at 24 h), reduce uptake by the RES (35% lower) and improve tumor accumulation (41% higher by analysis of gold content in tumor) effectively compared with the conventional irreversible system. Furthermore, preliminary study indicates that the system could be applied as computed tomography contrast agent in tumor imaging. The in vivo validity of the intelligent re-shieldable targeting system provides inspiration for the design of nanomaterials for cancer diagnosis and treatment.

1. Introduction

The diagnosis and treatment of cancer is a challenging problem. The non-specific tissue biodistribution made conventional chemotherapy have limited therapeutic effect and caused severe side effects on normal tissues [1,2]. Thus, increasing the accumulation of imaging probe or drug at the tumor site and decreasing the unwanted uptake by normal organs or the immune system are key factors for diagnosing and treating cancer. For the benefits of diverse sizes, architectures and surface properties, drug delivery system based on nanomaterials such as liposomes, micelles, and inorganic nanoparticles has emerged as a potentially useful tool to solve the problem [3]. Both passive targeting [4,5] (via the enhanced permeability and retention effect, EPR) and active targeting [6–8] could enhance the retention of nanoparticles in tumors and decrease the non-specific tissue biodistribution. However, the immunogenicity, hydrophobicity and exogenous origin of the targeting ligand, such as folate [9], antibodies [10] and peptides [11], would inevitably trigger the recognition and elimination of nanoparticles by the reticuloendothelial system (RES), thus impeding further accumulation of the nanoparticles in tumors. To solve the dilemma, programmed ligand targeting strategy was constructed. Specifically, the targeting ligand was shielded by protecting coronas such as polyethylene glycol (PEG) corona [12,13] or a caging group [14,15] in the bloodstream circulation to enhance the stability of nanoparticles. Upon reaching the tumor site, the “mask” will be removed by endogenous stimuli, such as pH [16] and enzymes [17], or exogenous stimuli, such as light [18,19] and temperature [20], to expose the targeting ligand for the uptake of nanoparticles by tumor cells. Frustratingly, not all the nanoparticles that arrived at the tumor site were effectively internalized by the tumor cells [21–23]. The nanoparticles that were not taken up by the tumor cells and returned to the bloodstream were already deshielded. However, because the protective coronas are often directly connected to the ligand or the nanoparticle surface via a chemical bond, it is difficult to shield the ligand again after the shielding coating is removed, thus facilitating nanoparticle recognition and capture by the RES. Therefore, the re-shielding of the unmasked nanoparticles in the bloodstream is urgently required to further prolong the circulation and help increase tumor enrichment.

The studies on reversible ligand shielding have already been performed. Donald E. Brooks et al. reported a pH and thermosensitive...
choline phosphate-based delivery platform that can reversibly bind to cells at pH 6.8 but not at pH 7.4 in vitro [24]. Although the ligand shielding strategy that based on thermosensitive polymer [25–29] or "popping up" ligand [30–32] have the capability of reversibly shielding ligand in theory, the further evaluation or application in vivo is still lacking. Our previous work showed that a shieldable tumor targeting system based on the pH-responsive self-assembly/disassembly of gold nanoparticles (Au NPs) could be obtained when simultaneously modifying tertiary amine molecules and the ligand on the surface of Au NPs [33,34]. The Au NPs assemble at pH 7.4 to shield the ligand inside the assembly structure. Then the active targeting ability of the ligand will re-activate for tumor cellular uptake when the Au NPs disassemble at the acidic microenvironment (pH 6.8) that is considered to be characteristic of the tumor extracellular pH [35,36]. Importantly, the assembly/disassembly process was reversible, thus endowing the system with great potential for applications. However, to the best of our knowledge, there are no related reports yet about the evaluation or application of reversible ligand shielding strategy in vivo so far.

In the present work, for the first time we proved the superiority of the intelligent re-shieldable targeting system (i.e., the reversible ligand shielding system) for improved tumor accumulation in vivo (Scheme 1). The system is based on the pH-responsive self-assembly/disassembly of gold nanoparticles modified with liver tumor targeting ligand glycyrrhetinic acid (denoted as Au NPs@Re-GA), GA is used as a ligand for the liver targeting because the GA-receptors abundantly existed on hepatocyte membranes [37]. Our previous studies have confirmed that GA could target liver cells especially liver tumor cells rather than receptor-negative cells [38,39]. As a control, an irreversible ligand shielding system based on pH-sensitive polyethylene glycol (PEG) detachable Au NPs (denoted as Au NPs@irRe-GA) was constructed (Supporting Information, Scheme S1 and Fig. S2). First, we examined the pH-Responsive assembly/disassembly of Au NPs@Re-GA by transmission electron microscope (TEM) and dynamic light scattering (DLS). We also conducted the cytotoxicity and cellular uptake assessment of Au NPs@Re-GA. The bloodstream circulation stability of the Au NPs@Re-GA by pharmacokinetics assessment and the recognition and elimination of Au NPs by RES were also conducted. Significantly, the tumor targeting capability of the intelligent re-shieldable targeting system by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) was performed. Furthermore, the system as computed tomography (CT) contrast agent for tumor imaging was preliminarily investigated. The in vivo validity of the intelligent re-shieldable targeting system in the present work provides inspiration for the design of nanomaterials for cancer diagnosis and treatment.

2. Materials and methods

2.1. Materials

Chloroauric acid was purchased from Energy Chemical Co. (Shanghai, China) Trisodium citrate dihydrate was purchased from Alfa Aesar Co. (Tianjin, China) α-Lipoic acid (LA) and 2-aminoethylisopropylamine were purchased from Adamas Reagent Co. (Shanghai, China). N-hydroxysuccinimide (NHS), N,N′-dicyclohexylcarbodiimide (DCC), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), succinic anhydride, 4-dimethylamiopyridine (DMAP) and 1-hydroxybenzotriazole (HOBT) were purchased from Aladdin Industrial Co. (Shanghai, China). NH2-PEG3.4k-OH, PEG2k, and PEG10k were purchased from Seebio Biotech, Inc. (Shanghai, China). Glycyrrhetinic acid (GA) was purchased from Fujie Pharmaceutical Co., Ltd. (Xi’an, China). N,N′-carbonyldimidazole (CDI) was purchased from J & K Scientific Ltd. (Beijing, China). 4-Formylbenzoic acid was purchased from Tianjin SanJiang Chemical Technology Co., Ltd. (Tianjin, China). NaOD and DCI solution were purchased from Dibai Corporation (Shanghai, China). Ethylenediamine was purchased from Tianjin chemical reagents supply and marketing company (Tianjin, China). Kunming mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The water used was prepared using Millipore Elix System (Millipore, Bedford, MA). All the materials and solvents were used as received without further purification.

2.2. Measurements

1H NMR spectra were collected on a Bruker AVANCE III 400 MHz spectrometer. ESI-MS was performed on LCQ-Advantage (ThermoFinnigan). Dynamic light scattering tests were performed with a Zetazizer Nano ZS90 instrument (Malvern Instruments Ltd., UK). The morphology and size of Au NPs were examined on a Tecnai G2 F20 transmission electron microscope (FEI, USA). Computed tomography experiments were conducted on a GE Discovery 750 HD medical system at Tianjin Hospital. The mole concentration of [Au] (atomic concentration) was measured by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, PerkinElmer Optima 8300).

2.3. Synthesis and characterization of materials

2.3.1. Synthesis of Au NPs

Au NPs (20 nm) were prepared according to the literature [40] with slight modifications. A sodium citrate tribasic dihydrate solution (prepared using deionized water, 5.88 × 10−3 M, 15 mL) was poured into a boiling solution of chloroauric acid (prepared using deionized water, 1 × 10−3 M, 150 mL). The solution color rapidly turned from bright yellow to black and gradually to wine red under vigorous stirring for...
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