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Near-infrared light triggered drug delivery system for higher efficacy of combined chemo-photothermal treatment

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ABSTRACT

The combination of chemotherapy and photothermal therapy is a promising strategy for cancer treatment. In the present study, indocyanine green (ICG), a widely used near-infrared (NIR) dye in photothermal therapy, and chemotherapeutic drug-doxorubicin (DOX) were loaded within the nanoparticles of novel designed arylboronic ester and cholesterol modified hyaluronic acid (PPE-Chol$_1$-HA), denoted as PCH-DI. We take advantage of reactive oxygen species (ROS) production capability of ICG and ROS-sensitivity of arylboronic ester to realize controllable drug release. It was confirmed that PCH-DI exhibited remarkable photothermal effect and light-triggered faster release of DOX with NIR laser irradiation. DOX in PCH-DI/Laser group exhibited the most efficient nucleus binding toward HCT-116 colon cells *in vitro*. Furthermore, enhanced cytotoxicity and promoted tumor growth suppression effect of PCH-DI on HCT-116 tumor xenograft nude mice and AOM-induced murine orthotopic colorectal cancer model was achieved under NIR laser irradiation. Thus, the co-delivery system based on PCH appears to be a promising platform for the combined chemo-photothermal therapy in tumor treatment.

Statement of Significance

In case of chemo-photothermal combination therapy, the synchronism of treatments plays an important role in achieving expected antitumor efficiency. In this study, a light triggered ROS mediated drug delivery system was developed with the help of ROS-sensitive moieties of arylboronic ester and ROS producer of ICG. We innovatively make use of the ROS production capability of ICG under NIR laser irradiation to promote a faster release of DOX resulting from swelling of PCH-DI due to the presence of arylboronic ester. Intracellular ROS detection demonstrated that ROS level of PCH-I increased under irradiation. Moreover, the faster release behavior of DOX from PCH-DI with NIR laser irradiation was confirmed by the *in vitro* drug release and cellular uptake study. Meanwhile, local hyperthermia was verified by photothermal effect tests. Therefore, the synchronism of the combination therapy was achieved via light triggered faster release of DOX (chemo-therapy) and local hyperthermia (thermal-therapy) using PCH-DI under irradiation. It was reasonable to attribute the efficient anti-tumor efficiency of PCH-DI both *in vitro* and *in vivo* to the enhanced synergistic effect of chemo-photothermal combination therapy with realization of synchronism. To this end, this novel co-delivery system has provided a promising solution for achieving the synchronism of treatment to strengthen the efficiency of combination therapy.

1. Introduction

Thermotherapy has been widely employed as a supplementary treatment for cancer [1]. The combination of chemotherapy and thermotherapy has great potentials in enhancing therapeutic efficacy and lowering adverse effects [2]. Recently, several studies have been focused on the mechanism of the combined chemo-photothermal therapy [3,4]. On the one hand, hyperthermia enhanced tumor sensitivity to chemotherapeutics as well as cellular uptake of drugs by affecting the permeability of cell membrane via lifting the environment temperature [5]. On the other hand, the photosensitizer that widely used as light-heat converting material in photothermal therapy generated reactive oxygen species (ROS) under NIR irradiation, which destabilized the endo-/lysosomal membrane and facilitated endo-/lysosomal escape [6]. Both of the above mechanisms are related with the synchronized

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execution of chemotherapy and photothermal therapy. In order to obtain the most efficient synergistic effect of combination therapy, administration sequences should be taken into consideration. It was presented that simultaneously performed chemotherapy and hyperthermia would achieve most valid therapeutic effects [7]. However, drug release and distribution *in vivo* cannot be precisely synchronized to photothermal therapy which is controlled by external laser irradiation. Therefore, a delivery system that possesses the characteristic of light triggered delivery is of greatly desire to achieve synchronism in chemo-photothermal therapy.

It is common to take advantage of stimuli responsive drug release systems, such as pH, enzymatic reactions and redox responsive drug carriers to realize microenvironment triggered drug release in tumor cytoplasm [8–11]. These strategies mainly take advantages of specific tumor microenvironments to facilitate drugs releasing from nanocarriers on demand. However, these stimuli strategies cannot coordinate the external laser stimulation and internal drug release in chemo-photothermal therapy. Thus, a new kind of stimuli method is called for in this system.

ROS is an important biological parameter related with the cellular redox state including superoxides (O^{2-}), hydroxyl radicals ($OH^{\cdot}$), peroxynitrites ($ONOO^{-}$) and hydrogen peroxides (H_2O_2), etc [12]. In consideration of the ROS generation by photosensitizer under NIR irradiation [13], it might be feasible to take advantage of ROS as an internal trigger signal in realizing synchronism of chemotherapy and photothermal therapy. It was reported that the arylboronic esters and its derivatives could be inserted with oxygen in the presence of ROS and followed by hydrolysis leading to cleavages of arylboronic esters [14]. Therefore, it was reasonable to employ the arylboronic esters as the ROS-sensitive component for site-specific delivery of imaging and therapeutic agents in response to ROS *in vivo* [15].

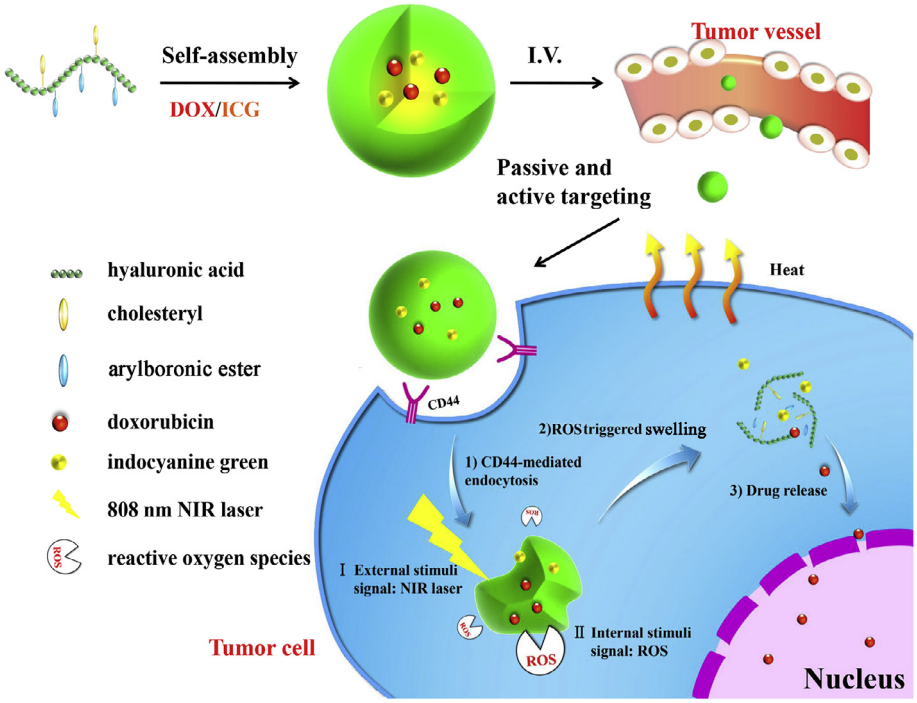
Chemotherapeutic agent doxorubicin (DOX) and photosensitizer indocyanine green (ICG) are extensively adopted as the model drugs in chemo-photothermal therapy, and both have been approved by the Food and Drug Administration (FDA) [16,17]. However, the clinical practice was limited to some extent by the undesired properties of drugs, such as short blood half-time and instability [18,19]. Recent years, typical work utilized the nano-based delivery systems for co-delivery of DOX and ICG, possessed great potential in combined chemo-photothermal therapy with the advantages of increased tumor accumulation of nanoparticles (NPs) and enhanced tumor regression [20]. Hyaluronic acid (HA) was a natural ligand of CD44 that overexpressed by various tumor cells [21]. The nanocarriers based on hydrophobically modified HA were widely studied, in which HA was employed as both hydrophilic skeleton and active targeting constituent [22].

In this study, we synthesized an arylboronic ester modified HA based NPs with DOX and ICG co-encapsulated. ICG not only played the role as receiver of NIR signal for heat-induced disruption, but also induced the rapid increase of intercellular ROS level, fabricating the light triggered drug delivery systems combined with the ROS-sensitive materials, which eventually realized the enhanced efficiency of chemo-photothermal therapy against colon cancer (Scheme 1). This work reported the rational design and relative *in vitro*/*in vivo* data for the first time.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (HA, MW 10 kDa) was purchased from Shandong Freda Biochem Co., Ltd. (Shandong, China). Cholesteryl chloroformate (Chol), 1-Ethyl-3 (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole monohydrate (HOBT) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). 4-(Hydroxymethyl) phenylboronic acid pinacol ester (HPPE), 1,1′-carbonyldiimidazole (CDI), 4-(dimethylamino)pyridine (DMAP), ethylenediamine (EDA), tetrabutylammonium iodide, pyrene and azoxymethane



Scheme 1. Illustration of self assembly, tumor targeting and light triggered ROS responsive nanosystem swelling and fast intracellular delivery of drugs for chemo-photothermal combination therapy. Briefly, nanoparticles underwent CD44 receptor mediated endocytosis. Under external NIR irradiation, the intracellular temperature and ROS level were sharply increased via the photoactive ICG. The increased ROS synchronously promoted the rupture of arylboronic ester bond leading to the light triggered drug release. The cytotoxicity of DOX cooperated with thermal toxicity of ICG leading to synergistic inhibition effects.