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Catalase-loaded cisplatin-prodrug-constructed liposomes to overcome tumor hypoxia for enhanced chemo-radiotherapy of cancer

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ABSTRACT

Aiming at improved therapeutic efficacies, the combination of chemotherapy and radiotherapy (chemo-radiotherapy) has been widely studied and applied in clinic. However, the hostile characteristics of tumor microenvironment such as hypoxia often limit the efficacies in both types of cancer therapies. Herein, catalase (CAT), an antioxidant enzyme, is encapsulated inside liposomes constituted by cisplatin (IV)-prodrug-conjugated phospholipid, forming CAT@Pt (IV)-liposome for enhanced chemo-radiotherapy of cancer. After being loaded inside liposomes, CAT within CAT@Pt (IV)-liposome shows retained and well-protected enzyme activity, and is able to trigger decomposition of H_2O_2 produced by tumor cells, so as to produce additional oxygen for hypoxia relief. As the result, treatment of CAT@Pt (IV)-liposome induces the highest level of DNA damage in cancer cells after X-ray radiation compared to the control groups. In vivo tumor treatment further demonstrates a remarkably improved therapeutic outcome in chemo-radiotherapy with such CAT@Pt (IV)-liposome nanoparticles. Hence, an exquisite type of liposome-based nanoparticles is developed in this work by integrating cisplatin-based chemotherapy and catalase-induced tumor hypoxia relief together for combined chemo-radiotherapy with great synergistic efficacy, promising for clinical translation in cancer treatment.

1. Introduction

Chemotherapy and radiotherapy are two mainstream cancer treatment modalities in clinic for various types of cancers [1–3]. However, severe side effects and inducible resistance associated with those conventional cancer therapies have made them difficult to satisfy the clinical requirements, obliging scientists to develop new strategies in cancer treatments. The chemo-radiotherapy, which is delivered by concurrent administration of chemotherapy and radiotherapy, has received tremendous interests in both basic research and clinical trials, aiming at improving therapeutic outcomes for tumor patients [4–6]. It is known that the chemotherapy in the concurrent chemo-radiotherapy could not only act as a radiosensitizer to promote radiotherapy efficacy, but also potentially eradicate those distant micro-metastases spared from the radiation beam exposure, leading to synergistic treatment outcomes especially for those advanced cancer patients [7–9].

However, like many other cancer therapeutics, chemo-radiotherapy is also not omnipotent to any types of cancers [10–12]. Similar to single radiotherapy, which needs sufficient molecular oxygen to stabilize radiation-induced DNA breaks and shows limited efficacy to kill tumor cells in the hypoxic regions within solid tumors, the therapeutic efficacy of chemo-radiotherapy has also been found to be significantly hindered by tumor hypoxia, a hostile characteristics of most solid tumors [13–17]. Recently, tumor hypoxia relief has been demonstrated to be a rather promising strategy for improved cancer radiotherapy [18–26]. Apart from increasing the tumor reoxygenation by the normalization of tumor vasculature or intratumoral oxygen delivery with artificial blood substitutes (e.g. perfluorocarbon) to overcome hypoxia-associated radiation resistance, several different groups including ours have uncovered that the decomposition of endogenous hydrogen peroxide (H_2O_2) inside the tumor with

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specific catalysts could be an alternative strategy for effective tumor reoxygenation [27–33]. More recently, we found that catalase, an enzyme that could decompose H_2O_2 into H_2O and O_2, could be efficiently encapsulated inside tantalum oxide (TaO_x) nanoshells, obtaining catalase loaded TaO_x nanoshells to efficiently relieve the tumor hypoxia by decomposing tumoral endogenous H_2O_2, subsequently leading to greatly improved cancer radiotherapy [34]. To date, however, efficient cancer chemo-radiotherapy with tumor hypoxia relief, preferable delivered by biocompatible nano-carriers, remains to be developed to our best knowledge.

Cisplatin, one of several robust chemotherapy drugs for efficient cancer chemo-radiotherapy in clinic, has recently been found to be able to be easily oxidized to cisplatin prodrug (cisplatin (IV)) by introducing two additional axial ligands for the purpose of decreasing cisplatin associated side effects [9,35,36]. In a recent work by our group, we uncovered that cisplatin (IV) pro-drug conjugated phospholipid together with other commercial lipids could easily form Pt (IV)-liposomes, which showed efficient tumor passive accumulation after intravenous (i.v.) injection [37]. To uncover whether tumor hypoxia relief could efficiently improve the therapeutic efficacy of chemo-radiotherapy, in this work, water soluble catalase (CAT) is encapsulated inside the liposomes formed with cisplatin (IV) pro-drug conjugated 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (Pt (IV)-DSPE), 1,2-dipalmitoyl-snglycero-3-phosphocholine (DPPC), cholesterol, and polyethylene glycol (PEG) conjugated DSPE (DSPE-$mPEG_{5k}$) (Fig. 1). It is found that catalase loaded inside such CAT@Pt (IV)-liposome shows retained and well-protected enzyme activity. As a result, such CAT@Pt (IV)-liposome induces the most effective DNA damage after being concurrently treated with X-ray radiation under the hypoxic condition. Like other PEGylated stealth liposomes, our CAT@Pt (IV)-liposome after i.v. injection shows efficient passive accumulation in tumors, in which the hypoxic status could be obviously relieved. Thereafter, the in vivo combined chemo-radiotherapy with CAT@Pt (IV)-liposome results in the most effective inhibition effect on tumor growth.

2. Experimental section

2.1. Materials

Catalase solution (≥35,000 units/mg protein) was purchased from Aladdin. Cisplatin was purchased from Beijing ZhongShuo Pharmaceutical Technology Development Co., Ltd. DSPE and DPPC were purchased from Xi'an Ruixi Biological Technology Co., Ltd. DSPE-$mPEG_{5k}$ was purchased from Laysan Bio Inc. Cholesterol was purchased from *J&K* Scientific Ltd. *N*-(3-Dimethylaminopropyl)–*N*-ethylcarbodiimide hydrochloride crystalline (EDC), *N*-Hydroxysuccinimide (NHS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were all purchased from Sigma-Aldrich. RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific Inc. All other chemicals were purchased from China National Pharmaceutical Group Corporation and used without further purification.

2.2. Preparation of liposomes

The DSPE-Pt (IV) was synthesized according to our previously developed method [37]. The dried lipid mixture of DSPE-Pt (IV) (16.66 mg), DPPC (10.48 mg), Cholesterol (2.87 mg) and DSPE-$mPEG_{5k}$ (5 mg) at the molar ration of 8:8:4:1 were firstly dissolved in 1 ml chloroform and then dried under vacuum. Afterwards, the dried lipid was dispersed in 2 mL phosphate buffered saline (PBS) containing 3 mg CAT (mass ratio: Pt: CAT = 0.9) for hydration. After being extruded through a 200 nm polycarbonate filter for 20 times, excess CAT was removed from liposomes by Sephacryl S-300 high resolution column (GE Healthcare).

2.3. Liposome characterization

The dynamic light scattering (DLS) measurement was carried out with a Malvern Zetasizer (Nano Z90). The morphology of

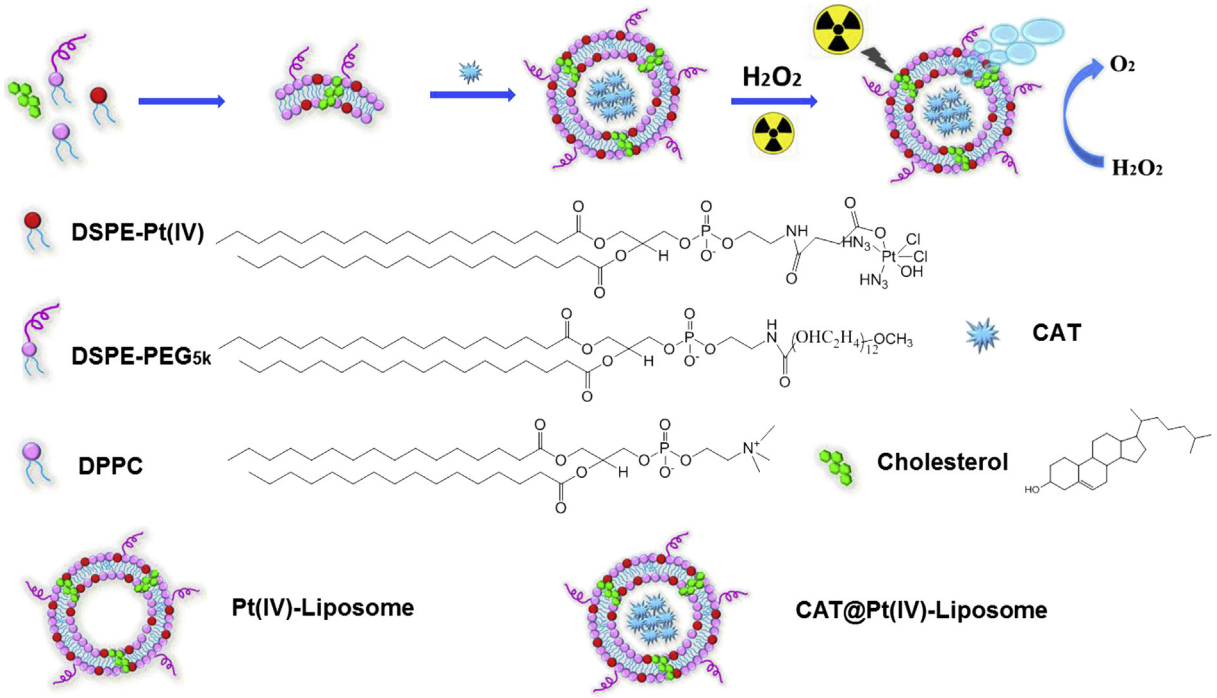

Fig. 1. A scheme showing the preparation of CAT@Pt (IV)-liposome for tumor hypoxia relieved cancer chemo-radiotherapy. The molar ratio of DSPE-Pt (IV), DPPC, cholesterol and DSPE-PEG was 8:8:4:1.