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Sensitivity to antitubulin chemotherapeutics is potentiated by a photoactivable nanoliposome

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ABSTRACT

Anti-microtubule therapy represents one of the most strategic cancer therapeutics. Tublin inhibitor such as paclitaxel (PTX) is well known to disturb the dynamic nature of microtubules, being considered as the first-line drug for various malignancies. However, PTX does not show favorable clinical outcomes due to serious systemic toxicities and low selectivity. The development of PTX delivery systems and combinational therapies has been conducted to enhance PTX efficacy with poorly defined mechanisms. Herein, we introduced a reactive oxygen species producible composite liposome based on a new photosensitizer sinoporphyrin sodium (DVDMS) to enhance the therapeutic effect of PTX through photochemical stimulation, and more importantly, the pivotal molecular regulation mechanisms were specifically explored. Compared with DVDMS-liposome (DL) or PTX-liposome (PL), the composite liposome DVDMS-PTX-liposome (PDL) exhibited a superior anti-tumor advantage following laser irradiation against MCF-7 breast cancer. The localized PTX release after PDL administration greatly decreased the drug dosage and laser power required, leading to much higher safety and lower costs. In vitro, the combined treatment significantly suppressed cell viability and potentiated cell apoptosis. The apoptotic central regulator Mcl-1 as a favorable target, was evaluated in association with photochemically enhanced sensitivity to anti-tubulin chemotherapeutics. Phosphorylation of Mcl-1 led to its direct degradation with the proteasome system, making it relatively unstable and potentiating cell death resulting from photochemical synergy via PDL plus laser irradiation. Further, a decrease in ATP production and glycolysis after PDL plus laser would prevent the possible energy-switch and apoptosis-escape by PTX alone treatment, thereby resulted in increased cell death in combinational therapy. Systemic administration of PDL followed by *in vivo* photochemotherapy achieved significantly improved therapeutic effects compared to either alone. And, the intrinsic fluorescence of DVDMS facilitated real-time imaging of PDL in tumors. Therefore, the present strategy with details at the molecular regulation could be a promising platform for antitublin chemotherapeutics.

1. Introduction

Anti-microtubule therapy represents one of the most strategic cancer therapeutics [1]. Microtubules participate in multiple cellular activities, such as cell division, cell motility, cell metabolism, etc. Microtubule targeting agents interfere with the microtubule function and arrest cells in mitosis, eventually leading to cell death [2]. The tubulin inhibitors in particular taxanes, paclitaxel continue to be clinical focus for various cancers. Paclitaxel (PTX) is a well developed microtubule stabilizer, binding to tubulin protein to induce microtubule polymerization and cell cycle arrest [3]. Although prolonged cycle arrest leads to initiation of apoptosis, which often suffer from the lack of tumor specificity and drug resistence [4,5]. Hence, the development of PTX delivery systems, including the use of liposomes, polymeric nanoparticles, micellar dispersions, and cyclodextrin complexes, have attracted

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the biopharmaceutical experts for developing efficient tumor targeting [6,7]. Liposomal formulations represent the optimization of PTX delivery, with Lipusu® (liposomal PTX approved by the State FDA of China) being recently used in clinical applications [8]. This conventional preparation is composed of phospatidylcholine and phosphatidylglycerol. Nevertheless, despite a considerable reduction in the side effects, the tumor cell killing efficacy of PTX was not equally improved [9]. Therefore, the challenge still remains to enhance cancer cell sensitivity to PTX and simultaneously reduce undesirable side effects.

Nanoscale drug delivery systems (DDS) have been developed to improve targeting drug delivery, exploiting the inherent passive tumor accumulation and the regulatable potential for drug release at the specific sites of interest [10,11]. An appealing approach is the use of light responsive DDS [12–14], through which spatiotemporal drug release can be controlled in response to a wide range of light wavelengths; this can be achieved by intracellular optogenetics [15] or through the external loading of light-sensitive compounds such as porphyrins [16]. The co-loading of a porphyrin without the use of genetic engineering or chemical reactions is a particularly safe and convenient route to externally activate drug release [17]. Previous studies have mainly focused on the site-specific light responsive drug release from various polymeric micelles as well as the synergistic combination of chemo- and phototherapy [18–21] leading to enhanced therapeutic effects with reduced drug dosages, suggesting a potential clinical cancer treatment platform. Pasparakis [22] utilized the first generation photosensitizer hematoporphyrin entrapped in bio-degradable polymers to exert photo-induced photochemotherapy. Similar designs have been made by others to sensitize chemotherapeutics [23], although the photochemical internalization and photolysis were proved, the molecular details regarding photochemotherapy remain unclear, which greatly restrict their further development.

In fact, few papers have addressed how and in what ways the loaded anti-cancer agents would be sensitized in a light-controlled DDS. Antitubulin agents, such as PTX and vincristine, target microtubules and thus block mitotic progression [24]. Following a prolonged mitotic arrest, cells typically face two fates: they either die in mitosis via apoptosis, or they undergo a process known as slippage, whereby they exit mitosis without division [25]. Identifying how the balance between apoptosis and slippage can be tipped in favor of death, thereby sensitizing cancer cells to antimitotic drugs, remains unaddressed. Bcl-2 familiarly proteins are crucial regulators of cell apoptosis [26]. Studies indicate that the anti-apoptotic members such as Bcl-2, Bcl-xl, Mcl-1, may confer resistance to antitubulin agents in tumors and promote tumor cells to evade apoptotic pathway through cellular upregulation [27]. Recent advances indicate Mcl-1 is an important regulator of mitotic cell fate [28], and the different phosphorylation sites of Mcl-1 would result in a distinct cell fate. In the prolonged mitotic period induced by microtubule stabilizing compounds, increased Mcl-1 expression enables the cells to survive. Conversely, inhibition of Mcl-1 has been shown to induce apoptosis [29]. Although Mcl-1 is known to oppose cell death, precisely how it functions in response to photodynamic therapy and combined photo/chemotherapy is poorly understood. In the present study, a new PEGylated liposome simultaneously co-loaded with PTX and an excellent sensitizer used in photodynamic therapy (PDT), sinoporphyrin sodium (DVDMS) [30,31], was designed (named as PDL) (Fig. 1a). We showed the photochemical catalysis provided potential to improve the efficacy of PTX through phosphorylation of Mcl-1 and subsequent degradation, thus enhancing mitochondrial-dependent cell apoptosis, which might be a useful strategy for overcoming the serious clinical problem of paclitaxel resistence in malignant tumors.

Moreover, studies also indicate that energy switching contributes to mitotic slippage and limits the success of mitotic-targeted therapies [32,33]. Following microtubule poison treatment, an AMPK-dependent increase in glucose uptake and generation of lactate was observed to support cell survival [32]. Mcl-1 is also involved in bioenergetics, which may link mitochondria oxidative phosphorylation and glycolysis besides its role in apoptosis regulation [34]. Yet, what possible relation between cellular energy supply, mitochondria integrity, and Mcl-1 expression needs exploration in this light responsible DVDMS-PTX nanoliposomes. Therefore, we further investigated the functional relevance of mitochondrial damage by studying the rates of mitochondrial respiration (oxygen consumption rate, OCR) and glycolysis (extracellular acid release, ECAR), aiming to explore the metabolic alterations occurring after PDL with laser treatment. The findings may provide new insights into the synergy of photo-antitubulin therapies.

2. Experimental section

2.1. Materials

1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-methoxy (polyethylene-glycol)-2000 (DSPE-PEG-2000), 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1, 2 -dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). PTX were obtained from Zhejiang HISUN Pharmaceutical Co., Ltd (Hangzhou, China). DVDMS (98.5% purity) was kindly provided by Professor Qicheng Fang from the Chinese Academy of Medical Sciences (Beijing, China). 4′, 6-diamidino-2-phenylindole (DAPI), 2-deoxy-D-glucose and oligomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2, 7-Dichlorodihydrofluo-rescein-diacetate (DCFH-DA) was from Molecular Probes Inc. (Eugene, OR, USA). Guava Viacount Reagent was supplied by Guava Technologies (Hayward, CA, USA). An Annexin V-FITC Apoptosis Detection Kit was obtained from keygen technology co., LTD (Nanjing, China). In situ cell death detection (terminal deoxynucleotidyl transferasemediated dUTP nick-end labeling, TUNEL) kit was purchased from Roche. MG132 was purchase from ApexBio (Houston, TX, USA). GAPDH antibody was obtained from EarthOX (San Francisco, CA, USA). Antibodies raised against Cleaved caspase-3, Cleaved poly (ADP-ribose) polymerase (PARP), β-tubulin, β-actin, Mcl-1, phosphorylation of Mcl-1 and Bcl-xL were purchased from Cell Signaling Technology (MA, USA).

2.2. Preparation of liposomes

Co-encapsulated liposomes of PTX and DVDMS (PDL) were prepared through the thin film-hydration method [35]. Briefly, PTX was added into the mixture of DPPC/DSPE-PEG-2000/cholesterol/DOPC (molar ratio of 58:5:35:2) dissolved in chloroform, which was removed under a nitrogen flow until a thin lipid film was formed. The lipid film was further dried for over 4 h under vacuum and sonicated (40 kHz) for 10 min by hydration at 65 °C with phosphate-buffered saline (PBS, pH 7.4) containing DVDMS to obtain a final total lipid concentration of 10 mg/mL. After hydration, liposomes were extruded through a polycarbonate membrane (pole size: 100 nm) using a mini-extruder (Avanti Polar Lipids, Alabaster, AL). The untrapped free PTX and DVDMS were removed by size exclusion chromatography using a Sephadex G-50 column. The final liposomes were stored in tight containers at 4 °C in the dark until further use. A similar method was used to prepare PTX-liposome (PL) and DVDMS-liposome (DL), as described above.