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Synergistic effects of negative-charged nanoparticles assisted by ultrasound on the reversal multidrug resistance phenotype in breast cancer cells

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ABSTRACT

We have fabricated a negative-charged nanoparticle (Heparin-Folate-Tat-Taxol NP, H-F-Tat-T NP) with dual ligands, tumor targeting ligand folate and cell-penetrating peptide Tat, to deliver taxol presenting great anticancer activity for sensitive cancer cells, while it fails to overcome multidrug resistance (MDR) in MCF-7/T cells (taxol-resistant breast cancer cells). Ultrasound (US) can increase the sensitivity of positive-charged NPs thereby making it possible to reverse MDR through inducing NPs' drug release. However, compared with the negative-charged NPs, positive-charged NPs may cause higher toxic effect. Hence, the combination of negative-charged NPs and US may be an efficient strategy for overcoming MDR. The conventional procedure to treat with NPs followed by US exposure possibly destruct multifunctional NPs resulting in its bioactivity inhibition. Herein, we have further improved the operating approach to eliminate US mechanical damage and keep the integrity of negative-charged NPs: cells are exposed to US with microbubbles (MBs) prior to the treatment of H-F-Tat-T NPs. Superior to the conventional method, US sonoporation affects the physiological property of cancer cells while preventing direct promotion of drug release from NPs. The results of the present study displayed that US in condition (1 MHz, 10% duty cycle, duration of 80 s, US intensity of 0.6 W/cm^2 and volume ratio of medium to MBs 20:1) combined with H-F-T-Tat-T NPs can achieve optimal reversal MDR effect in MCF-7/T cells. Mechanism study further disclosed that the individual effect of US was responsible for the enhancement of cell membrane permeability, inhibition of cell proliferation rate and down-regulation of MDR-related genes and proteins. Simultaneously, US sonoporation on resistant cancer cells indirectly increased the accumulation of NPs by inducing endosomal escape of negative-charged NPs. Taken together, the overcoming MDR ability for the combined strategy was achieved by the synergistic effect from individual function of NPs, physiological changes of resistant cancer cells and behavior changes of NPs caused by US.

1. Introduction

Multidrug resistance (MDR) is a major clinical obstacle for cancer chemotherapy. Drug-efflux pumps P-glycoprotein (P-gp) encoded by MDR1 gene, breast cancer resistance protein (BCRP) and multidrug resistance protein1 (MRP1) are responsible for the development of MDR in cancer cells [1,2]. Recent decades have witnessed several attempts to explore positive-charged nanoparticles (NPs) based strategies to overcome MDR [3]. For example, positive-charged Pluronic nanoparticles can participate in the enhancement of drug cytotoxicity [4,5]; the addition of resistance modulators or surfactants to PACA nanocarrier with positive charge increases intracellular drug concentration thereby reversing MDR [6]. Since cell surface is negative, positive charged NPs easily enter into cells resulting in efficient cellular uptake. However, at the same time, they may cause higher toxic effect including increased pulmonary side effects, systemic toxicity on white blood cells and enhanced thrombophilia. On the contrary, negative-charged NPs can reduce nonspecific uptake in liver and spleen to

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some extent attributed to the electrostatic repulsion between negative-charged NPs and cellular surface of RES organs [7–9]. Hence, it is necessary to develop an efficient strategy for negative-charged NPs to enhance cellular internalization and reverse MDR. We have previously fabricated a negative-charged nanoparticle (H-F-Tat-T NP) with dual ligands (tumor targeting ligand folate and cell-penetrating peptide Tat) to deliver taxol presenting great cytotoxicity for cancer cells [9]. However, the prepared NP was effective in enhancing cellular internalization but failed to overcome MDR in MCF-7/T cells (taxol-resistant breast cancer cells).

In recent years, ultrasound targeted microbubble destruction (UTMD) has been a prospective strategy for drug delivery. US combined with MBs can enhance the intracellular drug delivery attributing to sonoporation of US and inertial cavitation of MBs [10,11]. In addition, US with appropriate parameters is capable of reducing cell proliferation, altering gene expression levels and inducing DNA damage [10–12]. Accumulating documents revealed that US could enhance the sensitivity of chemotherapy drugs in drug-resistance cells [12,13]. Moreover, drug-loaded NPs with positive charge have been employed to reverse MDR phenotype assisted by US [14–16]. However, to the best of our knowledge, there have been few reports on negative-charged NPs combined with US to reverse MDR. It is essential to investigate a strategy for H-F-Tat-T NPs with negative-charge to overcome MDR.

Mechanical damage of NPs may be caused by US if cells were treated with NPs followed by US exposure [17,18]. In addition, our previous study revealed that cellular uptake of cancer cells treated with H-F-Tat-T NPs followed by US exposure was lower than that of individual NPs. It indicated that the conventional procedure is unfavorable for dual-functionalized NPs because the mechanical damage of US possibly destructs NPs resulting in its bioactivity inhibition, especially for the function restriction of dual ligands. Herein, we improved the operation procedure to keep NPs integrity. In detail, the resistant cancer cells are exposed to US and MBs prior to the H-F-Tat-T NPs treatment. Compared with the conventional method [13–15], the adjuvant US modality affects cancer cells rather than directly destructing NPs, which in turn preserves its integrity and function of dual ligands.

Similar to US, Tat peptide exerts the ability to enhance cell membrane permeability [19]. In this study, we also examined the individual and synergistic role for dual ligands and US on reversing MDR, especially the indirect influence on NPs from US. In addition, endosomal escape of NPs and endocytic inhibition experiment were also estimated to investigate the mechanism of its anticancer ability. The present study may provide a novel and effective strategy to extend the application of negative-charged NPs for the reversal of MDR.

2. Experimental section

2.1. Materials

Heparin sodium (Mn = 1.25 kDa, 189 U/mg) was obtain from Sinopharm Chemical Reagent Co. (Shanghai, China). Oregon green488 cadaverine and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylin docarbocyanine perchlorate (DiI) were obtained from Invitrogen Co. (USA). 1-ethyl-3,3-dimethylaminopropylcarbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP) and N-hydroxysuccinimide (NHS) were purchased from Medpep Co. (Shanghai, China). TAT peptide of the sequence Tyr-Gly-Arg-Lys-Lys-Arg-Arg–Gln-Arg-Arg-Arg (MW 1560 Da) was synthesized by ChinaPeptides Co. Ltd. (Shanghai, China). Lipid MBs were donated by Department of Pharmacy (Nanfang Hospital, China) and their size distribution ranged from 2 to 10 μm. Anti-P-gp antibody, anti-BCRP antibody, anti-MRP1 antibody and goat anti-rabbit secondary antibody were purchased from Abcam (Cambridge, MA, USA) and enhanced chemiluminescence kit was purchased from Biyuntian Biotech (Beijing, China). All other chemicals and reagents were purchased from Sigma Co. (USA). Spectra/Por 3 Dialysis Membrane (MWCO 3500) was purchased from Pharmacia (Piscataway, NJ). Ultrapure water (Milli-Q, 18 M Ω) was used in the experiment.

2.2. Preparation of Heparin-Folate-Tat-Taxol NPs (H-F-Tat-T NPs)

H-F-Tat-T NPs was prepared by a previous method. Briefly, succinylated–heparin in dry DMSO was stirred by gentle heating. Some amount of Folate-NH_2, Tat peptide, EDC and NHS were mixed and reacted at room temperature for 24 h. The deionized water was then added and dialyzed in a dialysis membrane. The solution was then lyophilized and H-F-Tat obtained. H-F-Tat and taxol was mixed in dry DMSO at 35 °C for 6 h. The mixture was dialyzed by a dialysis membrane for 48 h. After the excess water was concentrated, H-F-Tat-T NPs was lyophilized and a light yellow powder obtained. Oregon labeled NPs and DiI entrapped into Oregon labeled NPs were synthesized in the similar procedure. Size distribution and zeta potential of NPs were measured at 25 °C by dynamic lighting scattering (DLS) using a Zetasizer Nano-Zs (Malvern Instruments, UK). The morphology of NPs was observed by Transmission electron microscope (TEM, Hitachi HC-1, 80 kV) following negative staining with phosphotungstic acid. The size and concentration of MBs were determined by Coulter Multisizer IIe (Beckman Coulter, USA).

2.3. Ultrasound setup

Ultrasound waves were generated from an unfocused single element transducer (Vermon SA, Tours, France) with a center frequency of 1 MHz, driven by a medical acoustic generator (Sonicmaster ES-2, OGGiken Co., Ltd., Okayama, Japan). Before exposed to US, the 6-well culture plate was placed on the surface of the double distilled water at the temperature of 37 °C. The US transducer was then placed at the bottom of the water bath pointing upward with its beam aligned axially with the culture plate, which was 1 cm from the transducer source. There was degassed water between the transducer and the plate (Fig. 1). US energy at 1 MHz was intermittently delivered at intensities (spatial peak pulse average acoustic intensity, I SPPA) of 0.6 W/cm^2 with 10% duty cycle for 80 s according to previous study [20]. In our experiments, after MBs added, cells were exposed to US immediately, then followed by the addition of NPs.

2.4. Cell culture

MCF-7 cell line and MCF-7/T cell line were obtained from Aiyan biological technology cooperation (Shanghai, China). MCF-7 cells were cultured in RPMI 1640 containing 10% fetal bovine serum and 100 units/mL penicillin/streptomycin. MCF-7/T cells were cultured in a complete RPMI 1640 medium containing 20 ng/mL taxol to retain their drug resistance in daily culture. All cell lines were cultured in humid environment containing 5% CO_2 at 37 °C.

2.5. Cytotoxicity assay

MCF-7 cells and MCF-7/T cells were seeded in 96-well plate at an initial density of 4×10^3 cells/well in 200 μL RPMI1640 containing 10% FBS at 37 °C in humidified 5% CO_2, respectively. After incubation for 24 h, the culture medium/per well was replaced with 200 μL fresh medium containing different concentrations of free taxol or H-F-Tat-T NPs. After 48 h, 25 μL of MTT solution (1 mg/mL) was added followed by incubation for 4 h. Then, the solution