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Near infra-red laser mediated photothermal and antitumor efficacy of doxorubicin conjugated gold nanorods with reduced cardiotoxicity in swiss albino mice

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Abstract

Development of a multifunctional drug delivering system without side effects and compromising its therapeutic efficacy is a major concern in anticancer research. Recently, we have developed and demonstrated doxorubicin conjugated gold nanorod (DOX@PSS-GNR) as a sustained drug delivery vehicle. Here, we investigate the biodistribution, antitumor and photothermal efficacy of DOX@PSS-GNR along with its potential impact on cardiotoxicity in Swiss albino mice. The studies revealed that the accumulation of Free DOX in myocardium was 4-fold reduced in DOX@PSS-GNR animals, which further minimizes its cardiotoxicity by decreasing cardiac injury *via* preservation of cardiac markers. Further, DOX@PSS-GNR exhibits effective antitumor efficacy against Dalton lymphoma ascites (DLA) as evidenced by cell cycle analysis, apoptotic signals and reduced tumor volume and weight. In addition, DOX@PSS-GNR exhibits higher photothermal response and dominates DLA growth upon 0.1 W/cm^2 laser irradiation. In conclusion, multifunctional DOX@PSS-GNR with improved therapeutic index and reduced cardiotoxicity represents a promising candidate for cancer treatment.

Key words: Doxorubicin; Gold nanorods; Antitumor efficacy; Photothermal therapy; Cardiotoxicity

Introduction

The essence of any drug delivery system relies on its therapeutic efficacy which resides on its site specific drug delivery without any toxicological side effects. Design and development of multifunctional nanoparticle mediated drug delivery system have gained substantial progress in the field of anticancer research.[1-3] Among the various drug delivery systems, polymeric nanoparticle system shows excellent therapeutic efficacy against a wide range of biomedical applications. In addition, inorganic nanoparticles such as Gold nanorods (GNRs) have impressively demonstrated excellent physical–chemical properties with improved biocompatibility in living systems.[4] The size and shape tunable GNR with distinctive longitudinal surface plasmon resonance properties improve its usage in cancer therapies including, photothermal ablation and *in vivo* imaging studies.[5,6]

Doxorubicin (DOX) is the most widely used anti-neoplastic chemotherapeutic drug in the treatment of various hematological malignancies and solid tumors.[7,8] Despite its therapeutic efficacy, DOX causes severe non-specific side effects, which include dose dependent cardiomyopathy and myelosuppression.[9-11] This dose dependent cardiotoxicity limits its clinical utilization, as it causes irreversible myocardial damage leading to dilated cardiomyopathy with congestive heart failures.[12] Although the exact mechanism of

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DOX induced cardiotoxicity is elusive, reports have been suggested that the patterns of DOX induced anticancer effect and cardiotoxicity mechanism were not same.[13] The various molecular mechanisms of DOX induced cardiotoxicity include oxidative stress, myofibrilar detioration, calcium dysregulation, high energy phosphate pool and endothelin-1 levels, ECM remodeling, ceramide accumulation, neuregulin and cannaboid signaling.[13,14] Although a plethora of reports exists on minimizing the cardiotoxicity of DOX, there is a paucity of reports on preserving its therapeutic efficacy as well

DOXIL, a liposomal form of DOX is currently used against various types of cancers, including soft tissue sarcomas and lymphomas.[15-17] Although DOXIL enhances systemic circulation and reduces cardiotoxicity, its shows additional side effects which include concentration gradient dependent non-specific release of the encapsulated drug from the carrier, infusion reactions and hypersensitivity.[18,19] Hence, improved drug delivery systems for DOX mediated cancer chemotherapy still remain a challenge. Recently we developed GNR mediated DOX delivery system (DOX@PSS-GNR) using poly (sodium 4-styrensulfonate) (PSS) with excellent biocompatibility and sustained drug delivering characteristics and found to be potent anticancer agent against human breast cancer cells *in vitro*.[20] Herein, we ascertain and explored the in vivo antitumor efficacy of multifunctional DOX@PSS-GNR along with potential impact on cardiac tissue. Thus the present study was carried out to assess the bio-distribution, in vivo antitumor potential and NIR laser mediated photothermal efficacy of DOX@PSS-GNR against Dalton lymphoma ascites tumor. In addition, the extent of DOX induced cardiotoxicity of DOX@PSS-GNR was compared to the Free DOX in Swiss albino mice employing ECG, cardiac biochemical marker enzyme and by histopathological analysis.

Methods

Synthesis and characterization of DOX@PSS-GNR

Monodisperse GNR was synthesized using the silver ion-assisted seed mediated growth method and were surface modified with anionic PSS as described in our previous report.[20] The detailed methodology for synthesis and characterization of DOX@PSS-GNR is provided in supporting information. For all dilutions, DOX@PSS-GNR was dispersed in phosphate buffered saline (pH = 7.4).

Animals and tumor models

Six to eight week old male Swiss albino mice (20-25 g BW) were purchased from IISC Bangalore, India and were maintained in the Bharathidasan University animal house facility in accordance with the guidelines of CPCSEA with free access to food and water *ad libitum*. Dalton lymphoma ascites (DLA) in Swiss albino mice were obtained from Amala cancer research centre, Kerala, India and were regularly passage and maintained in peritoneum of the mice as ascites form. For solid tumor induction, 3×10^6 cells were injected into the hind region of the mice and the tumor growth (diameter) was measured using Verniar caliper

Biodistribution of DOX@PSS-GNR

Eighteen animals were randomly divided into three groups containing 6 animals each and were fasted overnight before the experiment. Each group of animals received a single equivalent dose of Free DOX and DOX@PSS-GNR (200 μl, DOX concentration 5 mg/kg BW) through intravenous tail injection. Control group received the equivalent volumes of PBS. After 24 h, mice were sacrificed and the heart, liver, lungs, kidney, spleen, testis were removed, weighed and tissue homogenate in PBS was prepared using homogenizer. DOX accumulations in various tissues were quantified using Dichloromethane extraction method.[21] The quantification of DOX was assessed by measuring its fluorescence using spectrophotometer at an excited and emission wavelength of 480 and 590 nm. The experiments were done in triplicates and the results were presented in mean ± SD.

Antitumor efficacy of DOX@PSS-GNR

The *in vivo* antitumor efficacy of DOX@PSS-GNR was tested in 30 DLA tumor mice divided into 5 groups (n = 6). After 72 h of tumor injection, mice received 4 cycles of drug regime every 3 days up to 15 days [Free DOX, DOX@PSS-GNR (5 mg/kg/cycle) and GNR-PSS (10 mg/kg/cycle)]. An equivalent concentration of GNR-PSS was optimized as same as in DOX@PSS-GNR. Each formulation was prepared and diluted to the final volume of 200 μl, injected by tail vein injection. Control mice and tumor control group was maintained without any drug regime (200 μl saline/cycle only). Tumor progression was assessed by monitoring animal body weight changes every two days. After 14 days, animals were sacrificed and the ascetic tumor cells were isolated rapidly and assessed for cell viability using Trypan blue exclusion assay and subjected to apoptotic assays (Hoechst staining and FACS analysis).

Cardiotoxic assessment of DOX@PSS-GNR

The chronic cardiotoxicity of Free DOX and DOX@PSS-GNR was assessed in Swiss albino mice using ECG, cardiac enzyme analysis and histopathological analysis.[22-25] Animals were randomly divided into three groups (n = 6). Free DOX and DOX@PSS-GNR group received 4 cycles of DOX treatment (equivalent to 5 mg/kg BW) at one-week intervals by tail vein injection for a cumulative dose of 20 mg/kg BW. Control group received 4 cycles of 200 μl saline each time. The detailed materials and methods for cardiotoxic analysis are provided in supporting information.

In vitro photothermal therapy

The *in vitro* photothermal effects of different concentrations of GNR, GNR-PSS and DOX@PSS-GNR (10, 20, 30, 40 and 50 μg/ml) under 0.1 W/cm^2 NIR laser were determined in MCF-7 cells using MTT assay. Control cells were also maintained without any drug and/or photo treatments. And the cellular morphology of MCF-7 in the presence or absence of GNR, GNR-PSS, Free DOX and DOX@PSS-GNR under laser irradiation was also determined using fluorescent microscopy (Nikon Eclipse 80i).