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Can two-step ablation combined with chemotherapeutic liposomes achieve better outcome than traditional RF ablation? A solid tumor animal study

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Supplementary information

Animal model

To develop the breast (or liver) tumor model, 2×10^6 4T1 (or H22) cells were subcutaneously injected into the mammary fat pad of the mice. The 4T1 breast tumor model line was a well-characterized line that we had been using for over 10 years. For all experiments and procedures, anesthesia was induced with intraperitoneal injection of pentobarbital sodium (45 mg/kg, chemical reagent factory of Foshan, China). Animal housing

was on a 12h light-dark cycle. All mice were fed on standard food and water. All animals were sacrificed with an overdose of carbon dioxide in a CO2 chamber. Tumors were monitored every 2 or 3 days to measure the growth. Prior experiments [9,10] had documented that 7-15 mm diameter of tumor range was an appropriate tumor size for RFA. Accordingly, solid nonnecrotic tumors (as determined with ultrasound) were used in our study and tumors were included in study until they reached 10-12 mm. The accuracy of the final measurements was verified by the senior author (W.Y., with 12 years of experience), who was blinded to the treatment group.

Histological analysis

For drug penetration and accumulation, mice after treatment were sacrificed to harvest tumor samples, then the specimen slides of tumor samples were obtained for drug tissue penetration by fluorescence (DiD). Specimen slides were imaged at the periablational rim at 20×magnification and analyzed using Caseviewer software (3DHISTECH, Hungary) to determine drug penetration and fluorescence intensity. To assess the influence of moderate hyperthermia on the collagen and reticular fibers resulting in IFP, the tumor sections at the predetermined time point (2h after RFA) were subjected to Masson staining (Solarbio, USA) and Gordon–Sweets reticular fiber staining (Solarbio, USA). Ten fields of vision were selected randomly and observed using an optical microscope. Image J software was used to analyze the content of collagen and reticular fibers in each pathological section. For safety analysis, the mice were sacrificed by anaesthesia, and major organs (heart, liver, spleen, lung and kidney)

were harvested and fixed with formalin and then embedded in paraffin. 5-7μm sections were cut with a paraffin slicing machine, followed by staining with hematoxylineosin (H&E) dyes.

Intravenous administration

For intravenous administration, drug-loaded liposomes were injected slowly (0.2-ml dose per animal for 30 seconds via a 27-gauge needle) into the tail vein at the selected administration time. The PTX or DOX dose was 0.2 mg (1 mg/ml concentration preparation).

Preparation of PTX-loaded liposome and DOX-loaded liposome

A total of 4mg of cholesterol, 16.8 mg of Lipoid E 80, 6 mg of PEG-DSPE and 1 mg of PTX were respectively weighed, dissolved in 10 ml of chloroform. Uniform film was obtained by reduced pressure distillation for 30 minutes at 37°C. Then adding 1 ml phosphate buffer solution (PBS, pH 7.4), suspension was obtained by vortex for 30s. Finally, PTX-loaded liposome was obtained by ice bath probing for over 3 minutes.

Lipids were dissolved in chloroform and evaporated at 37°C under reduced pressure. The dried lipid film was hydrated with 123 mM ammonium sulfate for SSL-DOX respectively. The ammonium sulfate gradient was established through eluting blank liposomes with PBS (pH 7.4). The DOX-loaded loaded liposome was obtained by incubation at 37°C for 15 min.

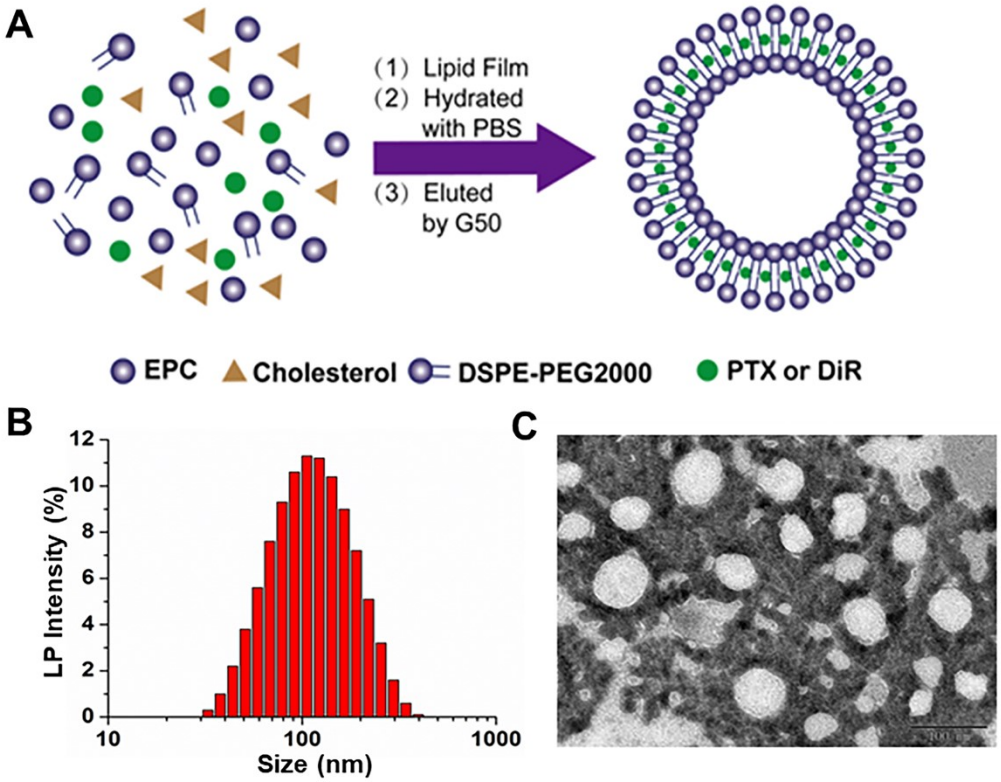

Figure S1. Preparation and characterization of liposomes. (A) The preparation procedure of liposomes. (B) The size distribution of liposomes by dynamic light scattering. (C) TEM image of liposomes. EPC, Cholesterol, DSPE-PEG2000: the phospholipid used to prepare liposomes.

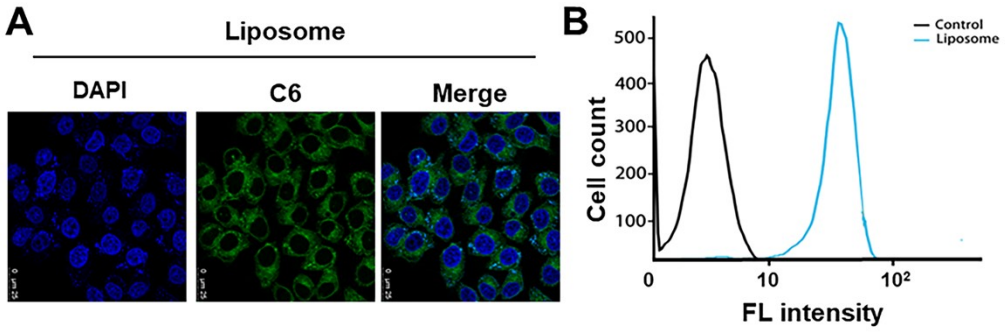

Figure S2. The cellular uptake in liposomes. (A) the confocal microscope image, (B) the flow cytometry. Confocal microscope and flow cytometry showed increased cellular uptake for liposomes (6.21±0.42 vs 12.65±1.42, p=0.003) compared with free drug.

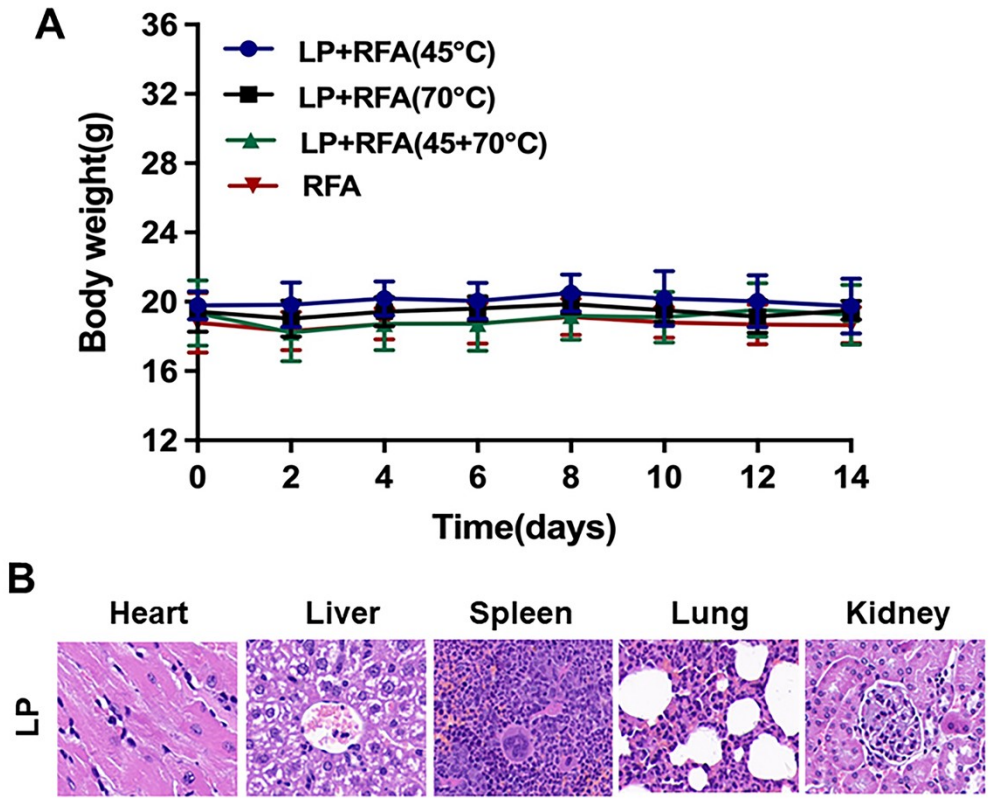

Figure S3. (A) The curves of body weight in different groups, the results showed that the difference of body weight among all groups has no significance. (B) The histological analysis of the main organs from mice treated with LP did not find any appreciable abnormality.