Co-delivery of TLR7/8 agonist and Dox using targeted liposome nanocarrier for bladder cancer therapy

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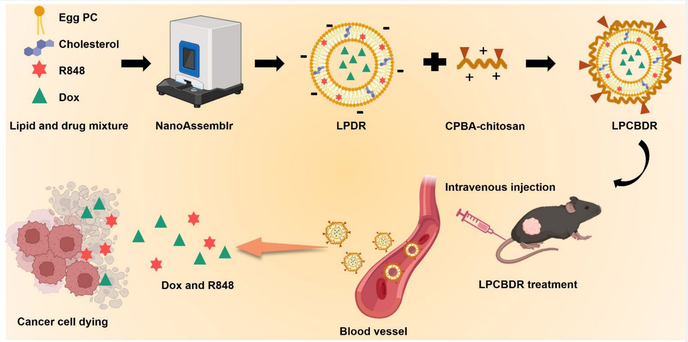
Introduction

Bladder cancer remains a significant global health burden, with 81,180 new cases and 61,700 deaths estimated in the U.S. in 2022 [1]. Chemotherapy and immunotherapy are standard cancer treatments, and their combination is increasingly used for tumor therapy. Liposome nanoparticles (LP NPs) enable the co-delivery of immunochemotherapeutic agent. Chitosan can enhance cellular uptake of NPs due to its cationic charge, while 4-carboxyphenylboronic acid (CPBA) selectively binds to sialic acid residues on bladder cancer cells. This study aims to develop a targeted liposome nanocarrier (Liposome-chitosan-CPBA, LPCB) for co-delivery of doxorubicin (Dox) and R848 for bladder tumor therapy.



Scheme 1. Dox and R848 loaded liposome-chitosan-CPBA nanoparticles (LPCBDR NPs) were firstly prepared using microfluidic method via the NanoAssemblr system. And then LPCBDR NPs were intravenously injected into MB49 subcutaneous tumor-bearing mice. LPCBDR NPs could actively target the tumor site and release Dox and R848. This targeted drug delivery method facilitated tumor cell eradication, thereby achieving a therapeutic effect.

Methods

LP NPs were prepared using a microfluidic method with egg phosphatidylcholine (eggPC), cholesterol, and R848 dissolved in ethanol, while Dox was dissolved in deionized water. LP NPs were then surface-modified with chitosan-CPBA and chitosan to obtain LPCB and liposome-chitosan (LPC) NPs. NPs were characterized by dynamic light scattering (DLS) and zeta potential analysis. Flow cytometry (FC) was used to test the targeting effect of coumarin-6 loaded LPCB or LPC NPs. Cytotoxicity of MB49 cells and dendritic cell activation of drug loaded LPCB were evaluated via CCK-8 and FC assays. For in vivo studies, C57BL/6 mice with subcutaneous MB49 tumors received tail vein injections of PBS, free Dox and R848 (FDR), Dox and R848 loaded LPC NPs (LPCDR), or Dox and R848 loaded LPCB NPs (LPCBDR)at day 0, 5, and 12, with tumor size monitored over time. On day 21, tumor and spleen tissues were collected. Tumor size and weight were recorded, and the tissues were enzymatically digested for the analysis of T/NK cell and antigen-presenting cell activity

References
[1] Siegel L, Miller D, Fuchs E, et.al. CA Cancer J Clin 2022: 7-33

Results

Preparation of LPC and LPCB nanoparticles

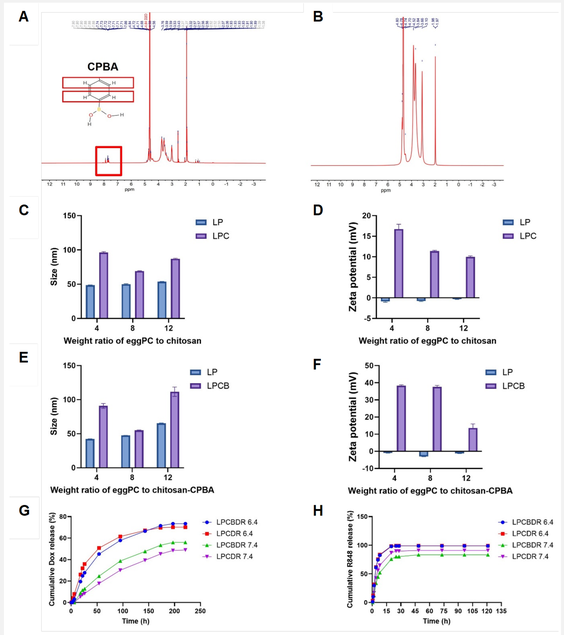


Figure 1. (A) H^1 NMR spectra of Chitosan-CPBA and (B) Chitosan. (C) Hydrodynamic size and (D) zeta potential of liposome (LP) and liposome-chitosan (LPC) NPs. (E) Hydrodynamic size and (F) zeta potential of liposome (LP) and liposome-chitosan-CPBA (LPCB) NPs. (G) Cumulative doxorubicin (Dox) release from Dox and R848 coloaded liposome-chitosan-CPBA (LPCBDR) and liposome-chitosan (LPCDR) NPs at different pH conditions (6.4, 7.4). (H) Cumulative R848 release from LPCBDR and LPCDR NPs at different pH conditions (6.4, 7.4).

In vitro targeting effect of LPCB NPs

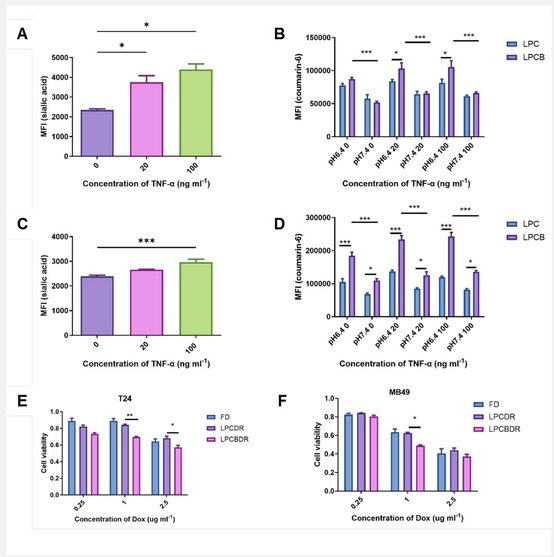


Figure 2. Mean fluorescence intensity (MFI) quantification of sialic acid expression levels on the surface of (A) T24 cells and (C) MB49 cells after incubation with different concentrations of TNF-α (0, 20, and 100 ng ml^{-1}) for 48h. MFI quantification of (B) T24 cells and (D) MB49 cells pre-treated with TNF-α for 48 hours, followed by a 1-hour co-incubation with coumarin-6-loaded LPCB and LPC NPs at 37°C at different pH (6.4, 7.4), pH 6.4 0, pH 6.4 20 pH 6.4 100 represents different concentrations of TNF-α (0, 20, and 100 ng ml^{-1}) at pH 6.4; pH 7.4 0, pH7.4 20, pH7.4 100 represents different concentrations of TNF-α (0, 20, and 100 ng ml^{-1}) at pH 7.4. Cytotoxicity of (E) T24 cells and (F) MB49 cells after treatment with different concentrations of Dox (0.25, 1, and 2.5 μg ml^{-1}) of FD, LPCDR, and LPCBDR NPs after 20 ng ml^{-1} TNF-α pre-treatment for 48h.

In Vivo Therapeutic Evaluation

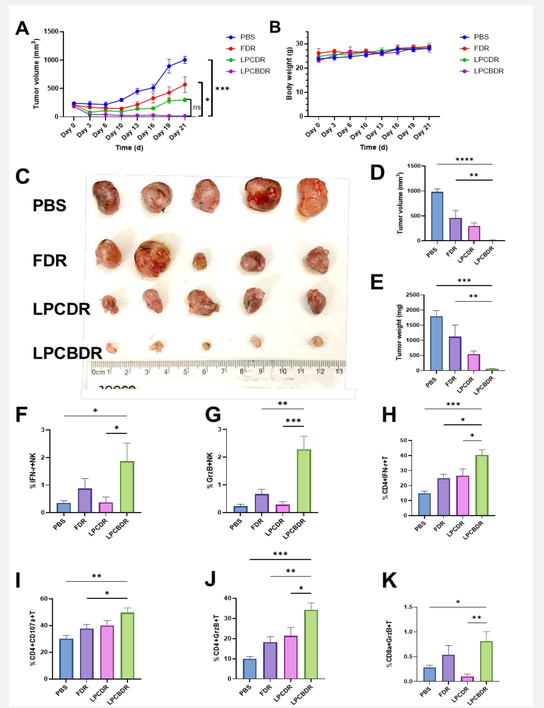


Figure 3. (A) Tumor growth curves of MB49 subcutaneous tumors in C57BL/6 mice (n = 5) treated via intravenous injection with PBS, free Dox and R848 (FDR), liposomal Dox and R848 (LPCDR), or targeted liposomal Dox and R848 (LPCBDR), each containing 20 μg Dox and 8 μg R848 in 100 μl PBS. (B) Body weight changes of mice during treatment in different groups. (C) Representative images of tumors harvested from each group on Day 21. (D) Tumor volumes and (E) tumor weights of tumors collected on Day 21. (F) Proportion of IFN-γ+ NK cells, (G) Granzyme B (GrzB)+ NK cells, (H) CD4+ IFN-γ+ T cells, (I) CD4+ CD107a+ T cells, (J) CD4+GrzB+ T cells, and (K) CD8a+ GrzB+ T cells in tumor tissues from different treatment groups.

Conclusion

In conclusion, this work successfully developed a novel targeted nanocarrier system co-delivering doxorubicin and R848 for synergistic chemo-immunotherapy of bladder cancer. The Dox and R848 co-loaded LPCBDR nanoparticles exhibited selective tumor targeting through CPBA-mediated recognition of sialic acid residues overexpressed on the surface of bladder cancer cells. Notably, the binding affinity between CPBA and sialic acid is enhanced under mildly acidic conditions, allowing LPCBDR nanoparticles to achieve stronger tumor cell targeting within the acidic tumor microenvironment. In vivo studies demonstrated that the targeted LPCBDR nanoparticles achieved significantly greater tumor suppression compared to non-targeted drug-loaded nanoparticles and free drugs. This dual-drug delivery system not only induced direct cytotoxicity but also synergistically activated both innate and adaptive immune responses within tumor tissue, resulting in superior therapeutic efficacy.

Acknowledgements
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