Electronic Supplementary Material

Ultralong Circulating Choline Phosphate Liposomal Nanomedicines for Cascaded Chemo-Radiotherapy

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Preparation and characterization of DOCPe and DOPC liposomes.

DOPC/cholesterol/DSPE-PEG2000 (2:1:0.16, molar ratio) or DOCPe/cholesterol/DSPE-PEG2000 (2:1:0.16, molar ratio) with a total mass of 2.5 mg were dissolved in 100 μL of chloroform and then briefly dried by N2. The dried lipid films were hydrated with 0.5 mL of 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) buffer (100 mM NaCl and 10 mM HEPES, pH 7.4) and extruded through two-stacked polycarbonate membranes (pore size = 100 nm, Whatman,
Buckinghamshire, UK) for 21 times. DiR-labeled liposomes were obtained by added 0.25% fluorescent dye of total mass of lipid.

Vorinostat-loaded liposomes were obtained by replacing fluorescent dye with 5% vorinostat of total mass of lipid. To load AQ4N, the lipid films prepared above were hydrated with aqueous solution containing 10 mg/mL AQ4N overnight and then extruded. The liposomes were washed three times by ultrafiltration tube with molecular weight cutoff 14,000 Da (Millipore, Bedford, MA) to remove free AQ4N. The size and surface charge of micelles were detected by a Zetasizer Nano ZS90 dynamic light scattering (DLS) system (Malvern Panalytical, Malvern, UK) with a He-Ne (633 nm) and 90° collecting optics. The data was analyzed by Malvern Dispersion Technology Software 7.0.2.

**Cells and animals.** 4T1 murine breast cancer cells from American Type Culture Collection (ATCC, Manassas, VA) were cultured in RPMI-1640 medium (Gibco, Waltham, MA) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco) at 37 °C in a 5% CO₂ atmosphere. BALB/c mice were purchased from Beijing HFK (Beijing, China). All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the University of Science and Technology of the Chinese Animal Care and Use Committee. To generate the 4T1 tumor model, 50 μL of 4T1 cells (2× 10⁴) in PBS containing 20% matrigel (BD Biosciences, Franklin Lakes, NJ) was implanted subcutaneously into BALB/c mice.

**Serum stability of DOCPe liposomes.** DOCPe liposomes were suspended in PBS (0.01 M, pH 7.4) containing 10% fetal bovine serum, then the size change was
monitored up to 48 h at predetermined time points by dynamic light scattering (DLS).

**MTT assay.** 4T1 cells were seeded into a 96-well plate (5,000 cells in 100 μL medium per well) and incubated overnight. Cells were treated with different formulations for 6 h then received 6 Gy radiation. Cells were incubated for another 72 h, and 25 μL MTT solution (5 mg/mL) were added to the medium. After another 4 h incubation, the medium was replaced by 100 μL DMSO and incubated for 10 min. The absorbance of the solution was measured at 570 nm using a Bio-Rad 680 microplate reader. The cell viability was normalized to that of cells cultured in the culture medium without any treatment.

**Colony formation assay.** 4T1 cells were seeded into a 24-well plate (800 cells in 2 mL medium per well) and incubated overnight. Cells were treated with different formulations for 6 h then received 6 Gy radiation. Cells were then incubated for 7 days. After removing the medium, cells were washed twice with PBS, and stained with 0.5% crystal violet (Sangon Biotech, Shanghai, China) in methanol for 30 min. Then, the cells were washed twice with distilled water before being visualized. The relative survival fraction was calculated by dividing the number of colonies of irradiated cells by the number of cells in control group. The survival fraction curves were generated via a nonlinear fitting using GraphPad 7.0.
**Figure S1.** UV-vis absorbance spectra of AQ4N, vorinostat, and DOCPe@(Vor+AQ4N) liposomes.

**Figure S2.** Time dependent change of size of DOCPe liposomes in PBS (0.01 M, pH=7.4) containing 10% fetal bovine serum at 37 °C.
**Figure S3.** Representative HE pictures of main organs of mice from various groups after tumor growth inhibition study.

**Table S1.** The drug loading capacity (m_{\text{drug}} / m_{\text{lipid}}, \%) of vorinostat and AQ4N in DOCPe liposomes.

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<tr>
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<th>Vorinostat</th>
<th>AQ4N</th>
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<tbody>
<tr>
<td>DOCPe@Vor</td>
<td>1.85 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>DOCPe@AQ4N</td>
<td>-</td>
<td>2.93 ± 0.46</td>
</tr>
<tr>
<td>DOCPe@((Vor+AQ4N)</td>
<td>1.76 ± 0.27</td>
<td>2.64 ± 0.26</td>
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