Supporting information for

Investigation of the intracellular oxidative stress amplification, safety and anti-tumor effect of a kind of novel redox-responsive micelles

1. Experimental section

1.1 In vivo pharmacokinetic and biodistribution studies

Eighteen SD rats were randomly assigned to three groups (n = 6 per group) according to the form of PTX administered (a single dose of 6 mg/kg i.v. through the tail vein): free PTX, FSST-PTX or FT-PTX. At time points of 0 (pre-dose), 0.083, 0.17, 0.5, 1, 2, 4, 8, 12 and 24 h post injection, blood (0.3 mL) was collected into heparinized polyethylene tubes via jugular veins and instantly centrifuged at approximately 4,000 rpm for 10 min followed by supernatant plasma (100 μL) collected and stored at -70 ºC prior to analysis by HPLC. Methanol (400 μL) was added to the rat plasma samples and stirred vigorously for 1 min. Then, the mixture was centrifuged at 10,000 rpm for 10 min. An aliquot of the upper organic phase (400 μL) was transferred to another tube and evaporated to dryness at 40 ºC with nitrogen. The resulting extract was dissolved in of methanol (150 μL) and mixed. After centrifugation at 13,000 rpm for 10 min, the supernatant (100 μL) was taken for HPLC analysis. Winnonlin 5.2 software was utilized to analyze the pharmacokinetic parameters: peak concentration (C$_{\text{max}}$), area under the plasma concentration-time curve (AUC), elimination half-life ($T_{1/2}$) and mean residence time (MRT) of PTX for each formulation.

For biodistribution studies, the xenograft MCF-7 tumor-bearing nude mice were intravenously injected with free PTX, FSST-PTX or FT-PTX at PTX dose of 10 mg/kg. At time points of 0.5, 2, 4, 8, 12 and 24 h post injection, blood samples were collected and mice were sacrificed by cervical dislocation. Tissues were excised and lightly rinsed with saline, weighed and stored at -20 ºC until assay. The concentration of PTX in plasma and homogenized tissues was determined by HPLC.
2. Results

2.1 Characterization of FSST and FT

Figure S1-S5 showed the \(^1\)H-NMR spectrum of Pluronic F127, F127-\(p\)-NPC, F127-APD, F127-APD-\(p\)-NPC, and F127-Cys. All the chemical shifts were expressed in parts per million (\(\delta\)) relative to the solvent signal.

\(^1\)H-NMR spectrum analysis of F127: \(^1\)H-NMR (600 MHz, CDCl\(_3\), \(\delta\)): 3.42-3.51 (m, 4H×196, CH\(_2\)CH\(_2\)O of PEO), 3.32-3.33 (m, 3H×67, CH\(_2\)CHO of PPO), 1.03-1.05 (t, 3H×67, CH\(_3\)) (Figure S1).

\(^1\)H-NMR spectrum analysis of F127-\(p\)-NPC: \(^1\)H-NMR (600 MHz, CDCl\(_3\), \(\delta\)): 8.27-8.36 (d, 2H, Ar H), 7.39-7.51 (d, 2H, Ar H), 3.39-3.64 (m, 4H×196, 3H×67, CH\(_2\)CH\(_2\)O of PEO and CH\(_2\)CHO of PPO), 1.13-1.15 (t, 3H×67, -CH\(_3\)). The peaks appeared at 8.27-8.36 and 7.39-7.51 belonged to the benzene ring of \(p\)-NPC (Figure S2).

\(^1\)H-NMR spectrum analysis of F127-APD: \(^1\)H-NMR (600 MHz, CDCl\(_3\), \(\delta\)): 4.24 (s, 1H, CH), 3.75-3.77 (t, 2H, CH\(_2\)), 3.71-3.73 (t, 2H, CH\(_2\)), 3.39-3.66 (m, 4H×196, 3H×67, CH\(_2\)CH\(_2\)O of PEO and -CH\(_2\)CHO of PPO), 1.13-1.15 (t, 3H×67, CH\(_3\)). New peaks appeared at 4.24 and 3.75-3.77, which were referred to the ortho-dihydroxy groups of APD. These peaks which belonged to \(p\)-NPC disappeared (Figure S3).

\(^1\)H-NMR spectrum analysis of F127-APD-\(p\)-NPC: \(^1\)H-NMR (600 MHz, CDCl\(_3\), \(\delta\)): 8.28-8.36 (d, 2H, Ar H), 7.39-7.51 (d, 2H, Ar H), 5.60 (s, 1H, CH), 4.31-4.44 (d, 2H, CH\(_2\)), 3.76-3.81 (d, 2H, CH\(_2\)), 3.40-3.65 (m, 4H×196, 3H×67, CH\(_2\)CH\(_2\)O of PEO and CH\(_2\)CHO of PPO), 1.13-1.15 (t, 3H×67, CH\(_3\)). The characteristic peaks of benzene ring appeared at 8.28-8.36 and 7.39-7.51 after being activated by \(p\)-NPC (Figure S4).

\(^1\)H-NMR spectrum analysis of F127-Cys: \(^1\)H-NMR (600 MHz, CDCl\(_3\), \(\delta\)): 5.61 (s, 1H, CH\(_2\)), 4.24 (s, 1H, CH\(_2\)), 3.72-3.76 (d, 2H, CH\(_2\)), 3.39-3.64 (m, 4H×196, 3H×67, CH\(_2\)CH\(_2\)O of PEO and CH\(_2\)CHO of PPO), 3.18-3.21 (d, 1H, CH\(_2\)), 2.59 (s, 1H, CH\(_2\)), 1.13-1.15 (t, 3H×67, CH\(_3\)). After the reaction with cystamine dihydrochloride, the amide bonds were formed. Thereby, the peaks of benzene ring disappeared and new peaks appeared at 3.18-3.21 and 2.59, which were attributed to the methylene protons near the disulfide bonds (Figure S5).
1H-NMR spectrum analysis of FSST: 1H-NMR (600 MHz, CDCl₃, δ): 5.60 (s, 1H, CH₂), 4.25-4.27 (t, 1H, CH₂OH), 4.20 (s, 1H, CH₂), 3.64-3.77 (m, 42H, CH₂CH₂O), 3.40-3.64 (m, 4H×196, 3H×67, CH₂CH₂O of PEO and CH₂CHO of PPO), 2.91-2.94 (t, 1H, CH₂), 2.78-2.80 (t, 1H, CH₂), 2.62-2.66 (t, 1H, CH₂), 1.96-2.08 (t, 3H, CH₃), 1.75-1.81 (m, 1H, CH₂), 1.22-1.52 (m, 10H, CH₂), 1.13-1.15 (t, 3H×67, CH₃), 0.83-0.87 (m, 5H, CH₃).

1H-NMR spectrum analysis of FT: 1H-NMR (600 MHz, CDCl₃, δ): 5.60 (s, 1H, CH₂), 4.25-4.27 (t, 1H, CH₂OH), 4.20 (s, 1H, CH₂), 3.64-3.77 (m, 42H, CH₂CH₂O), 3.40-3.64 (m, 4H×196, 3H×67, CH₂CH₂O of PEO and CH₂CHO of PPO), 2.83-2.87 (t, 1H, CH₂), 2.78-2.80 (t, 1H, CH₂), 1.96-2.08 (t, 3H, CH₃), 1.75-1.81 (m, 1H, CH₂), 1.22-1.52 (m, 10H, CH₂), 1.13-1.15 (t, 3H×67, CH₃), 0.83-0.87 (m, 5H, CH₃).

2.2 In vivo pharmacokinetic studies

The plasma PTX concentration vs. time profiles observed for PTX, FSST-PTX and FT-PTX were shown in Figure S7A. Two micelles achieved larger AUC and longer half-life compared to PTX (P < 0.01), while PTX was cleared from the circulation at 8 h after administration. FSST-PTX and FT-PTX significantly prolonged the in vivo circulation time of PTX. The pharmacokinetics parameters were summarized in Table S4. FSST-PTX and FT-PTX extended the elimination half-life (T₁/₂) of PTX from 2.78 h (PTX) to 7.29 h and 9.01 h, respectively (P < 0.01). Meanwhile, MRT values of FSST-PTX and FT-PTX were 3.67-fold and 4.97-fold higher than PTX, respectively (P < 0.01). Moreover, the AUC₀⁻∞ increased by about 2.58-fold for FSST-PTX and 3.44-fold for FT-PTX compared to PTX (P < 0.01).

The in vivo biodistribution of PTX, FSST-PTX and FT-PTX after intravenous administration was evaluated by quantitatively detecting the PTX concentration in different tissues. Figure S7B showed the comparison of AUC₀⁻二十四 among PTX, FSST-PTX and FT-PTX in various tissues. FSST-PTX and FT-PTX exhibited similar distributions in each organ. Their distribution in tumor were 1.75-fold and 1.68-fold higher than that of PTX. Moreover, their distribution in the heart, liver and spleen was
less than that of PTX, but higher in plasma than the latter.
Figure S1 The $^1$H NMR image of F127

Figure S2 The $^1$H NMR image of F127-$p$-NPC
Figure S3 The $^1$H NMR image of F127-APD

Figure S4 The $^1$H NMR image of F127-APD-$p$-NPC.
Figure S5 The $^1$H NMR image of F127-APD-Cys.
Figure S6 Different tissues collected after i.v. administration of different materials for H&E Staining. (A) male mice. (B) female mice.
**Figure S7** *In vivo* pharmacokinetic and biodistribution studies. (A) Plasma concentration-time curves of PTX, FSST-PTX and FT-PTX after i.v. administration to SD rats at the PTX dose of 6 mg/kg (mean ± SD, n=6). (B) The comparison chart of area under the curves (AUC$_{0-24}$) of PTX in the heart, liver, spleen, lung, kidney, plasma and tumor of the xenograft MCF-7 tumor-bearing nude mice following i.v. administration of PTX, FSST-PTX or FT-PTX at a PTX dose of 10 mg/kg for 24 h (mean ± SD, n=4). The unit of AUC$_{0-24}$ in plasma is h·μg/mL. The units of AUC$_{0-24}$ in other organizations is h·μg/g. **P < 0.01: significantly different from PTX.**
Figure S8 Histological study of tissues of heart, liver, spleen, lung and kidney after treatment by H&E staining (20×).
Table S1  Pharmacokinetic parameters of different PTX preparations after i.v. administration to SD rats at the same 6 mg/kg PTX dose (mean ± SD, n=6). **P < 0.01: significantly different from PTX group.

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<th>Parameters</th>
<th>PTX</th>
<th>FSST-PTX</th>
<th>FT-PTX</th>
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<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>17087 ± 2433</td>
<td>18972 ± 1567</td>
<td>18669 ± 1749</td>
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<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2.78 ± 0.39</td>
<td>7.29 ± 0.85**</td>
<td>9.01 ± 2.08**</td>
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<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;(h·ng/mL)</td>
<td>13158 ± 651</td>
<td>33974 ± 5136**</td>
<td>45327 ± 5143**</td>
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<tr>
<td>MRT (h)</td>
<td>1.80 ± 0.16</td>
<td>6.61 ± 0.87**</td>
<td>8.95 ± 2.47**</td>
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