SUPPORTING INFORMATION

Reduction responsive modification induced higher efficiency for attenuation of tumor metastasis of low molecular weight heparin functionalized liposomes

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1. ^{1}H-NMR of the synthesized conjugate of LMWH-DTSP-OSu

^{1}H-NMR of LMWH, LMWH-eda and LMWH-DTSP-OSu was shown in Fig. S1. The formation of LMWH-eda was confirmed with characteristic peak of δ (ppm) 3.00 (m, 2H, $CH_2N(H_2)$, ***a***). The formation of LMWH-DTSP-OSu was confirmed with characteristic peak of δ (ppm) 2.72 (s, 4H, Su, ***b***), 2.92 (t, 4H, $2CH_2S$, ***c***).

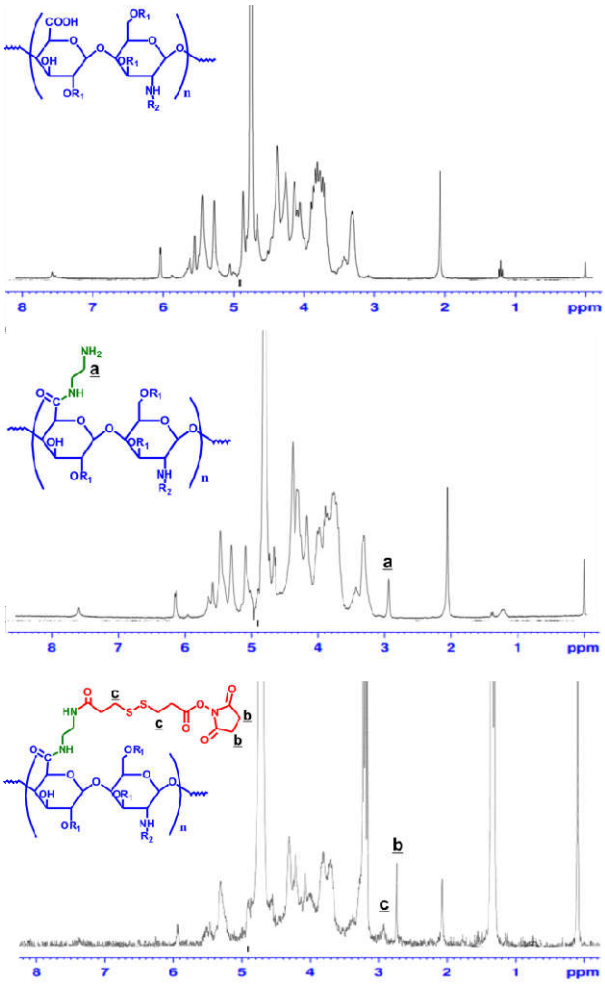

Fig. S1 ^{1}H-NMR of LMWH, LMWH-eda and LMWH-DTSP-OSu

2. Binding efficiency of LMWH

The binding efficiency of LMWH was evaluated with toluidine blue assay. As shown in Table. S1, the binding efficiency was all above 80 %, indicating the high modification efficacy. Along with the increase of feeding LMWH concentration,

ranging 0.5 to 2 mg/mL, the binding efficiency slightly decreased from ~ 90 % to ~ 80 %.

Table S1. Bing efficiency of LMWH with different feeding amount（$n = 5$）

Concentration of LMWH (mg/mL)	Concentration of LMWH on liposome (mg/mL)	Bonding efficiency of LMWH (%)
LMWH-DOX-Lip		
0.5	0.47 ± 0.01	93.20 ± 2.59
1.0	0.90 ± 0.03	89.90 ± 3.44
1.5	1.28 ± 0.04	85.60 ± 2.40
2.0	1.61 ± 0.03	80.60 ± 1.50
LMWH-ss-DOX-Lip		
0.5	0.46 ± 0.01	91.20 ± 2.59
1.0	0.90 ± 0.01	89.90 ± 0.74
1.5	1.27 ± 0.04	84.87 ± 2.72
2.0	1.60 ± 0.06	80.30 ± 2.91

3. Activeated partial thromboplastin time (APTT)

APTT assay was conducted to evaluate the blood plasma coagulation time. Briefly, 90 μL plasma mixed with 10 μL heparin, LMWH or LMWH-DTP-OSu solutions with LMWH concentrations of 2, 4, 6, 8 and 100 μg/mL. After equilibrating at 37 ℃ for 1 min and 7 min, 100 μL APTT reagent and 100 μL $CaCl_2$ (25 mM) were added respectively. Then record the plasma coagulation time. As shown in Table. S2, after chemical modification, the plasma coagulation time for LMWH-DTP-OSu was shorter than that of LMWH with same LMWH concentration, indicating chemical modification could lower the bleeding risk of LMWH.

Table S2. APTT assay for heparin, LMWH and LMWH-DTP-OSu

Concentration	APTT (s)			
(μg/mL)	Control	heparin	LMWH	LMWH-DTP-OSu
2	35.6	85.6	38.8	36.3
4	35.6	>120	43.4	37.1
6	35.6	-	50.6	37.8
8	35.6	-	59.5	38.4
100	35.6	-	>120	60.2

4. Blood routine examination

The C57BL/6 mice (healthy) were administrated with saline, DOX-sol, DOX-Lip, LMWH-DOX-Lip and LMWH-ss-DOX-Lip at a dose of 3.5 mg/kg DOX every third day. After 21 days, blood samples were collected for blood analysis.

As presented in Fig. S2, after intravenous injection of DOX-Lip, LMWH-DOX-Lip and LMWH-ss-DOX-Lip, white blood cell numbers, red blood cell numbers, blood platelet numbers and hemoglobin contents showed no significant difference compared with Saline group, indicating little blood toxicity.

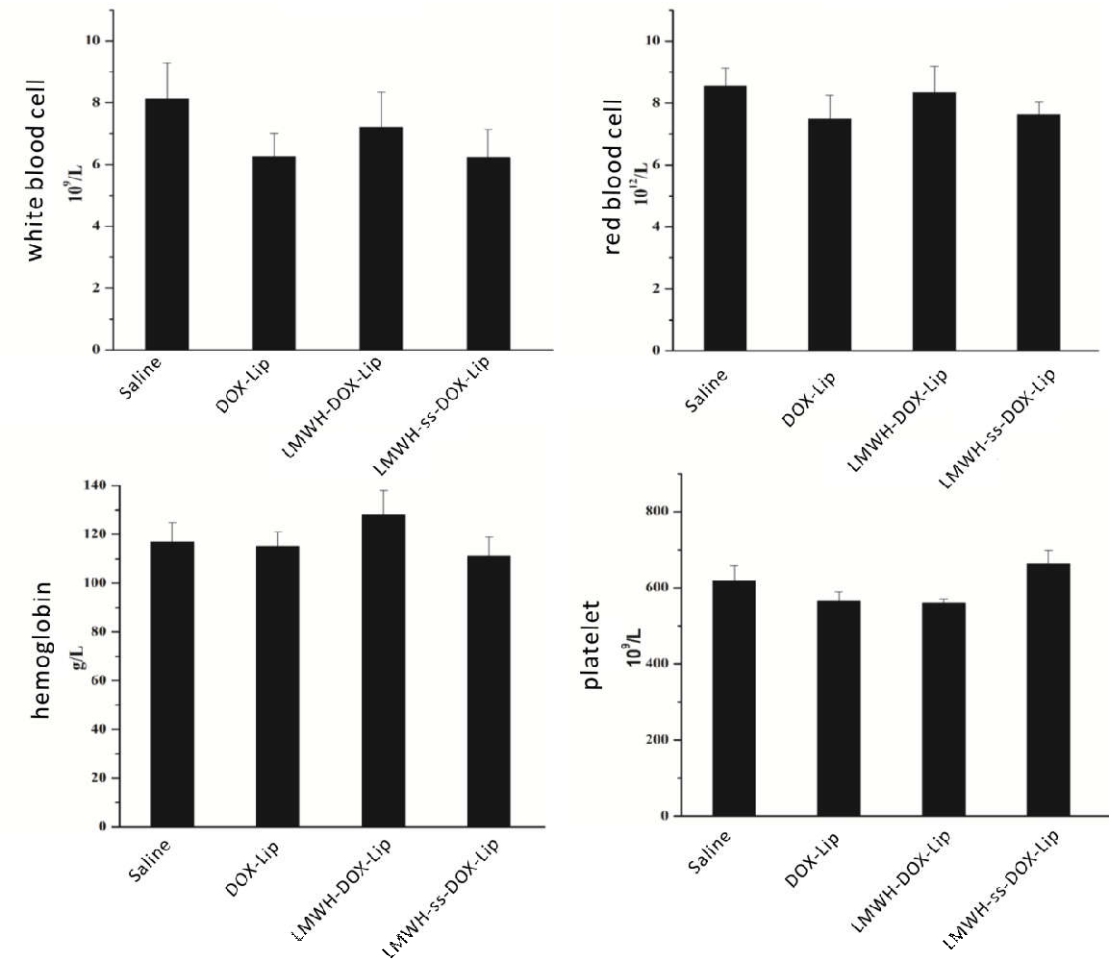

Fig. S2 Blood routine examination of white blood cell number, red blood cell number, blood platelet number and hemoglobin content of indicated groups.