Supporting Information for

Original article

Targeted delivery of hyaluronic acid nanomicelles to hepatic stellate cells in hepatic fibrosis rats

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Supporting figures

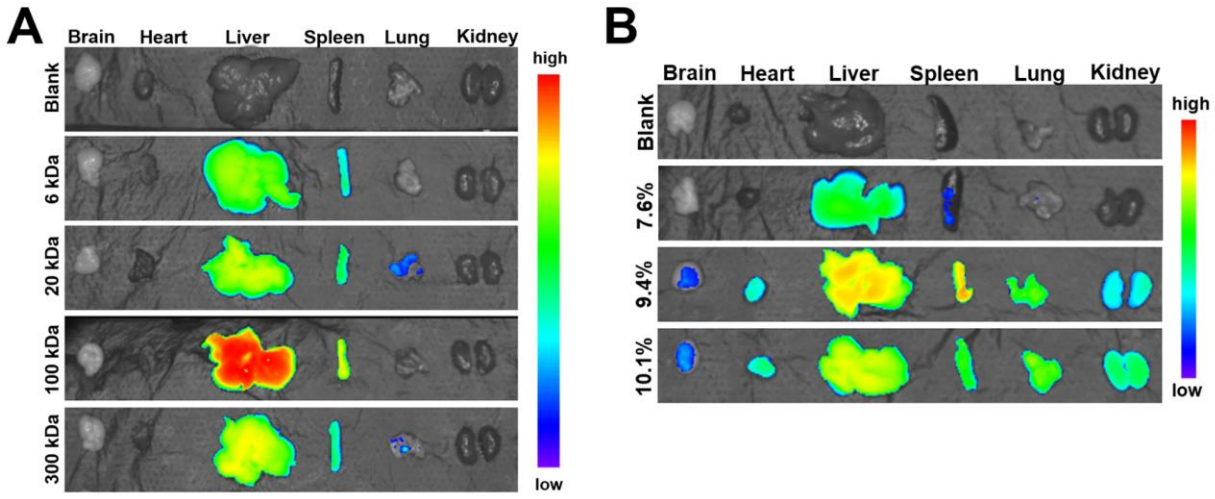

Figure S1 *Ex vivo* FITC fluorescence images showing the bio-distribution of (A) different molecular weight and (B) different modified degree of HA in mice at 15 min post-injection.

Comments to Figure S1. The *in vivo* distribution of HA is closely related to its molecular weight. HA of more than 1000 kDa is mainly distributed in the skin and plays a role in the protection and repair of skin; HA of 100–1000 kDa is mainly distributed in liver, kidney and spleen and other organs with CD44 receptor expression; HA of 10–100 kDa is mainly distributed in lymphoid tissues. Therefore, it is very important to select appropriate molecular weight of HA to prepare HA–DOCA for increasing the liver targeting of HA micelles. We used FITC to label the HA and identified suitable HA with the best liver targeting effect through *in vivo* tissue distribution experiments. The synthesis process of FITC-labeled HA (FITC-HA) was consistent with HA–DOCA. FITC-HA with different molecular weights (6, 20, 100 and 300 kDa) could be obtained by changing the molecular weight of HA. Male Kunming mice were randomly divided into four groups (n = 3). FITC-HA with different molecular weights was administered intravenously (FITC concentration was 1 mg/kg). At 15 min after

administration, the mice were sacrificed and the major organs (heart, lung, spleen, liver, and kidney) were excised. All organs were washed with physiological saline and dried with filter paper for *ex vivo* imaging of FITC fluorescence (Fig. S1A). The results showed that the HA of 100 kDa had the most obvious liver targeting effect, and we chose HA of this molecular weight to carry out the follow-up study.

The substituting degree of HA–DOCA also affects the characterization of HA micelles. The higher is the substituting degree of HA–DOCA, the better is its hydrophobicity. More side chains of HA–DOCA can be inserted into the hydrophobic core of HA micelles, so that the structure of HA micelles is more compact, thus reducing the particle size. However, the high substituting degree of HA–DOCA can destroy the original structure of HA, which may affect the liver targeting effect. Therefore, it is very important to select appropriate substituting degree of HA–DOCA for increasing the liver targeting of HA micelles. We used FITC instead of DOCA to label the HA (FITC-HA) and FITC-HA with different substituting degree (7.6%, 9.4% and 10.1%) could be synthesized by changing the feeding ratio between HA and FITC. The best substituting degree of HA conjugate was also determined by *in vivo* tissue distribution experiments. The results showed that 9.4% substituting degree of HA conjugate had the most obvious liver targeting effect (Fig. S1B), and we chose this substituting degree to carry out the follow-up study.

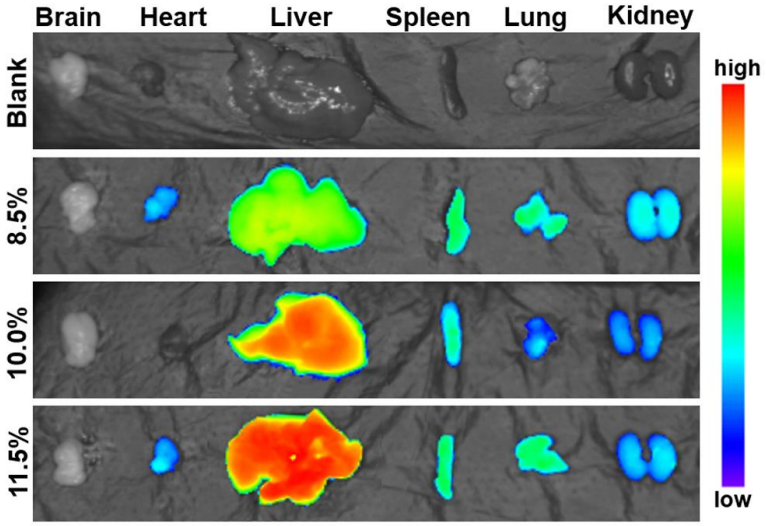

Figure S2 *Ex vivo* DiD fluorescence images showing the bio-distribution of different proportion of HA–DOCA in mice at 15 min post-injection.

Comments to Figure S2. We further researched the liver targeting ability of HA micelles prepared by different proportions of HA–DOCA in the prescription through *in vivo* tissue distribution experiments. The results confirmed that the best proportion of HA–DOCA in the prescription was 11.5% which had the most obvious liver targeting effect.

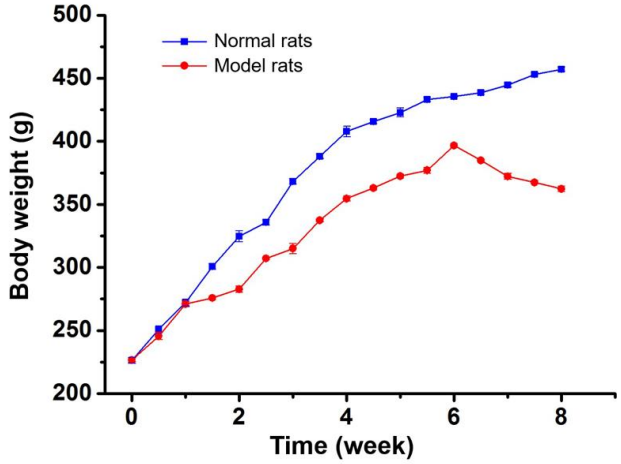

Figure S3 Changes in body weight of normal rats and model rats. Data represent mean ± SD (n = 5).

Comments to Figure S3. The body weight of normal rats was always on the rise, and the weight increased about 230 g after 8 weeks. However, the weight gain of model rats was significantly lower than that of normal rats, and even decreased after 6 weeks, indicating the occurrence of disease.

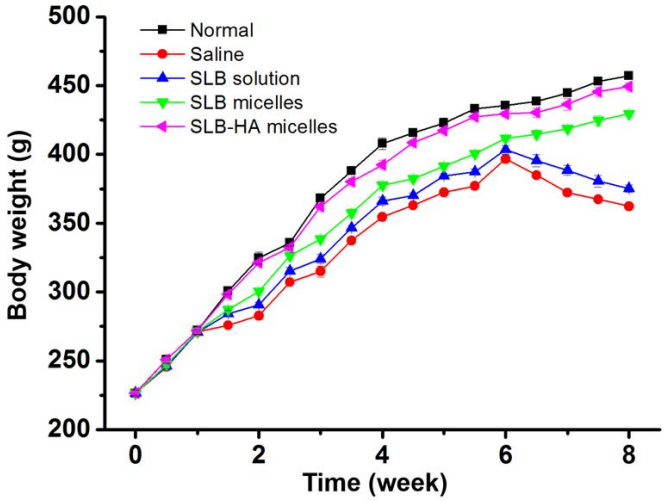

Figure S4 Changes in body weight of normal rats and fibrotic rats treated with physiological saline, SLB solution, SLB micelles and SLB-HA micelles. Data represent mean ± SD (n = 5).

Comments to Figure S4. Due to the occurrence of liver fibrosis, rats treated with saline had poor living conditions and slow growth of body weight. At the later stage of modeling, their body weight gradually decreased, indicating a deeper degree of fibrosis. The body weight of SLB solution group was slightly higher than that of saline group, but it still decreased after 6 weeks, indicating that SLB had some therapeutic effect on liver fibrosis but very limited. The body weight of SLB micelles group was further increased and there was no weight loss phenomenon, which indicated that SLB micelles could improve the anti-hepatic fibrosis effect. Compared with SLB solution group and SLB micelles group, the body weight of SLB-HA micelles group was significantly increased and was very close to the normal level, indicating the excellent anti-hepatic fibrosis effect of SLB-HA micelles.

Supporting tables

Table S1 Effect of feeding molar ratio on the characterization of micelles.

Feeding molar ratio of [phospholipids]/[DOCA-Na]	Particle size (nm)	PDI	The largest drug loading (%)
2:1	20.3	0.113	4.6
1:1	30.5	0.182	6.5
1:2	40.0	0.197	8.0
1:3	76.2	0.238	9.2
1:4	100.9	0.427	10.1

Comments to Table S1. With the increase of DOCA-Na ratio, the particle size of micelles increased, and the drug loading volume increased correspondingly. Taking into account the drug loading volume and particle size of the two factors, we chose 1:2 as the best feeding molar ratio of phospholipids/DOCA-Na to prepare micelles.

Table S2 Effect of HA–DOCA amount on the characterization of HA micelles.

Feeding amount of HA–DOCA (mg)	Proportion of HA–DOCA (%)	Particle size (nm)	Stability of HA micelles
5	8.5	30.1	Good
6	10.0	35.6	Good
7	11.5	42.2	Good
8	12.9	260.6	Bad
9	14.3	500.4	Bad

Comments to Table S2. The proportion of HA–DOCA in the prescription affected the characterization and liver targeting effect of HA micelles. If HA–DOCA was used too much, the particle size of HA micelles would be too large and the stability would be too bad. However, when HA–DOCA was used too little, the liver targeting ability of HA micelles was affected. Therefore, our aim was to use HA–DOCA as much as possible without affecting the particle size and stability of HA micelles. The results showed that the best proportion of HA–DOCA in the prescription was 11.5%.

Table S3 Blood and biochemical index of physiological saline and blank HA micelles.

Parameters	Physiological saline	Blank HA micelles
WBC (10^9/L)	10.3±0.5	10.1±0.2
RBC (10^{12}/L)	8.8±0.4	8.9±0.3
Plt (10^9/L)	434±41.2	428±37.5
HGB (g/L)	158±7.3	152±6.4
HCT (%)	47.8±2.6	45.3±3.1
MCV (fL)	44.4±1.4	47.3±2.4
MCH (pg)	16.6±0.2	16.3±0.1
MCHC (g/L)	290±21.2	286±23.1
CK (U/L)	660.3±65.3	671.5±56.1
LDH (U/L)	548.8±55.4	556.2±57.2
AST (U/L)	121.4±11.2	117.3±9.8
ALT (U/L)	47.5±8.3	45.2±6.4
ALP (U/L)	137.8±11.6	141.3±11.1
BUN (mmol/L)	7.4±1.2	7.3±0.9
CREA (μmol/L)	11.6±2.2	12.3±1.4
TBIL (μmol/L)	1.5±0.2	1.6±0.3

WBC, white blood cell; RBC, red blood cell; Plt, platelet; HGB, hemoglobin; HCT, haematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CREA, creatinine; TBIL, total bilirubin.

Comments to Table S3. As liver targeting drug delivery carriers, the liver toxicity of HA micelles needed to be investigated emphatically. ALT, ALP and AST are representative biochemical indicators for evaluating liver function. The ALT, ALP and AST levels of rats treated with HA micelles were not significantly different from those treated with saline, indicating that HA micelles could not cause obvious toxicity to liver. The other blood and biochemical indexes of HA micelles group were also similar to those in the saline group, proving the good safety of HA micelles.