Supporting Information

Europium doped $NaYF_4$ nanoparticles cause the necrosis of primary mouse bone marrow stromal cells through lysosome damage

Kun Ge[a,b], Wentong Sun[a], Shaohan Zhang [a], Shuxian Wang [b]*, Guang Jia [a], Cuimiao Zhang [a], and Jinchao Zhang[a]*

Animals and treatment

4–5 weeks SPF（specific pathogen free) female ICR mice (18–20 g) were obtained from Beijing Vitalriver Experimental Animal Technology Co. Ltd. The mice were housed in plastic cages in a temperature-controlled and ventilated room. The conditions were maintained at 20 ± 2 ^{o}C room temperature, 60 ± 10 % relative humidity, and 12 h light/dark cycle. Sterilized commercial pellet diet and distilled water for mice were available ad libitum. All experiments were performed in compliance with the relevant laws and institutional guidelines, and also state that the Administration Office Committee of Laboratory Animal has approved the experiments. After one week acclimation, the mice were randomly divided into seven groups which include control group and six experimental groups: NY50 4.8

[a] Key Laboratory of Chemical Biology of Hebei Province, Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of the Ministry of Education, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, P. R. China.

[b] Affiliated Hospital of Hebei University, Baoding 071000, P.R. China.

*corresponding author: Tel: (86)312-5079627; E-mail: 13832200993@163.com; jczhang6970@163.com

mg/kg (NY50 low-dose group), NY50 24 mg/kg (NY50 middle-dose group), NY50 120 mg/kg (NY50 high-dose group), NY200 4.8 mg/kg (NY200 low-dose group), NY200 24 mg/kg (NY200 middle-dose group), NY200 120 mg/kg (NY200 high-dose group). Each group contained six mice. NY50 and NY200 suspension solutions were administered to each mouse of the experimental groups at 4.8, 24, and 120 mg/kg/day over a period of 35 days, and sterilized physiological saline with the equal volume was given to the control mice by intraperitoneal injection. The symptoms were observed over the period of administration such as changes in body weight, skin and fur, behavior pattern, circulatory, respiratory, urine, feces, and so on. Thirty five days later, the animals were sacrificed by anesthetization with ether. The blood was collected from ophthalmic veins. A part of blood was heparinized for haematological toxicity, and the remaining blood was centrifuged, then the serum was used for biochemical analysis. The vital organs of liver, spleen, and kidney were excised and weighed accurately.

Coefficients of liver, spleen and kidney

The body and organs such as liver, spleen, and kidney were weighted after thirty five days post-exposure. The coefficients of organs to body weight were calculated as the ratio of wet weight of organs (mg) to body weight (g).

Biochemical assay of serum

The serum was obtained by centrifugation for the whole blood (3,000 rpm,15 min). Alkaline phos-phatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum were measured by assay kits (Beijing Capital Medical University Clinical Science Center) for evaluating liver function. Urea, creatinine (Cr), and uric acid (UA) in serum were measured by assay kits (Randox Laboratories Ltd. UK) for evaluating nephrotoxicity. The enzymes of alpha-

hydroxybutyrate dehydrogenase (HBDH), creatine kinase (CK), and lactate dehydrogenase (LDH) in serum were measured using assay kits (Merit Choice Bioengineering (Beijing) Co,. Ltd.) for evaluating cardiac damage by an automatic biochemical analyzer (7600-110, Hitachi, Tokyo).

Blood-element test

The blood-element was analyzed by assay kits (Randox Laboratories Ltd. UK) by an automatic hematology analyzer (COULTER LH 750, Beckman, American). The blood-element included white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), blood platelet (PLT), red cell distribution width (RDW) , and corpuscular volume (CV).

Statistical analysis

The results were shown as mean ± standard deviation (SD). ‘‘Student–Newman–Keuls Multiple Comparisons Test’’ (ANOVA) was used for comparison between the experimental groups and the control group. *P* values less than 0.05 were considered statistically significant at the 95% confidence interval.

Results

Coefficients of liver, spleen, and kidney

The mice’s autonomic activities of the high-dose group decreased within 6 h after injection. There was no significant difference of body weights between the control and all experimental groups (Fig. S1). Mice were sacrificed at thirty-five day, and splenomegaly, hemorrhagic ascites, abdominal adhesion and some white particulates with tunicary deposited on liver, spleen, intestine and peritoneum were observed in several mice of NY50 and NY200 high-dose groups, but no abnormal change was

observed in other groups. As shown in Fig. S2, there was no obvious difference from the control for the coefficient of liver and kidney to body weight. However, the spleen in all tested groups showed significant higher coefficient than that of the control group.

Biochemical parameters in serum

The changes of biochemical parameters in the serum were presented in Table S1. There were no significant difference for all parameters in the NY50 and NY200 low-dose groups ($p>0.05$). NY50 and NY200 middle-dose groups increased AST level (NY50 and NY200: $p<0.05$) and AST/ALT (NY50: $p<0.05$, and NY200:$p<0.01$). For the high-dose groups, AST level (NY50 and NY200: $p<0.0001$) and AST/ALT (NY50: $p<0.0001$, and NY200: $p<0.01$) were also increased significantly.

In the high-dose groups, there were no difference in the value of CK, but the HBDH and LDH levels increased obviously compared with the control group (NY50: $p<0.0001$, and NY200: $p<0.01$).

Correspondingly, the levels of UA, Cr, and BUN in the blood are often used as the markers of renal toxicity. In all tested groups, there was no difference for the values of UA, Cr and BUN compared with the control group ($p>0.05$).

The blood-elements

The results were presented in Table S2, which showed that there were no significant difference for blood-elements in NY50 and NY200 low- and middle-dose groups, and NY200 high-dose group compared with the control group. But WBC number increased and HGB level decreased significantly in the NY50 high-dose group.

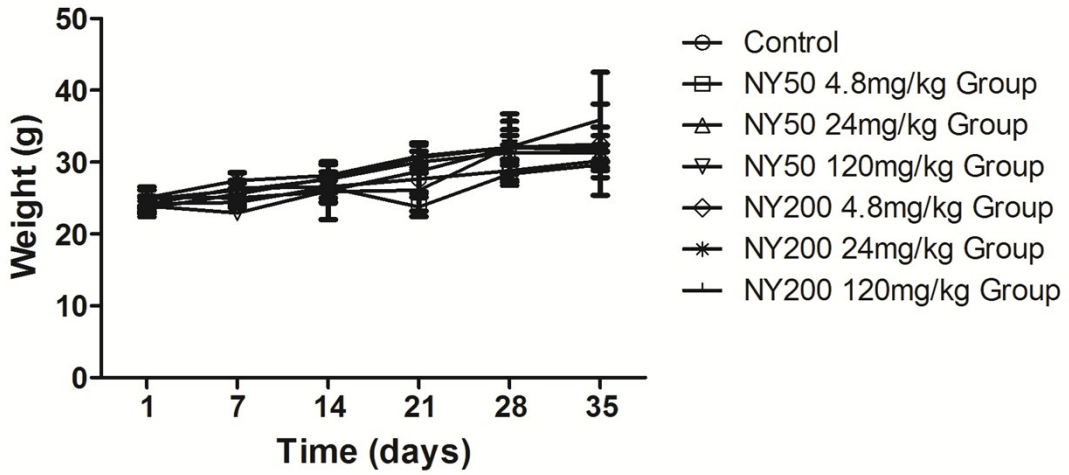

Fig. S1 Mice weight changes by intraperitoneal injection with NY50 and NY200 at 4.8, 24, and 120 mg/kg/day over a period of 35 days.

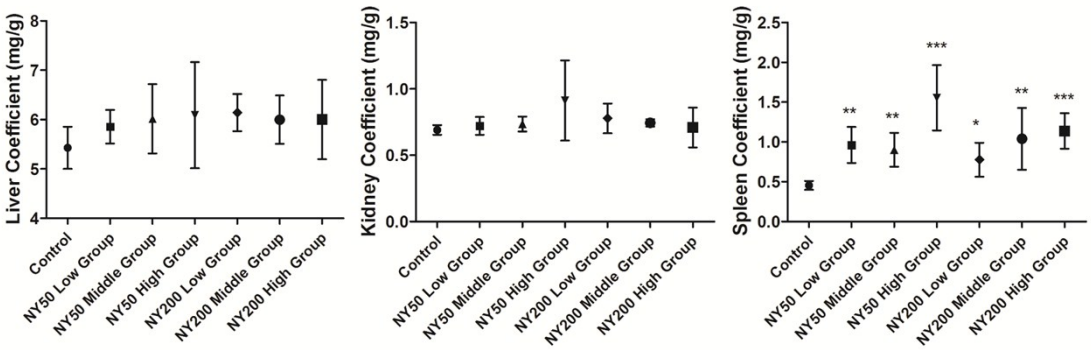

Fig.S2 Coefficients of liver, spleen, and kidney. NY50 Low-, Middle-, High-dose group represent NY50 4.8 mg/kg, NY50 24 mg/kg, and NY50 120 mg/kg respectively; NY200 Low-, Middle-, High-dose group represent NY200 4.8 mg/kg, NY200 24 mg/kg, and NY200 120 mg/kg respectively. (*$P<0.05$, **$P<0.01$ and ***$P<0.0001$ vs control)

Table S1 Changes of biochemical parameters in the serum of mice after intraperitoneal injection with $NaYF4:Eu^{3+}$ particles

Groups	Urea (mmol/L)	Cr (umol/L)	UA (umol/L）	CK （U/L）	HBDH （U/L）	LDH （U/L）	ALP （U/L）	AST （U/L）	ALT （U/L）	AST/ALT
Control	12.32±2.31	11.22±3.19	735.82±70.46	933.85±232.86	1023.82±91.37	3793.35±476.27	63.73±8.49	242.50±37.22	62.17±5.29	3.89±0.44
NY50 4.8mg/kg	12.84±1.18	12.48±1.73	749.77±59.65	656.72±86.63	1278.85±138.27	4902.19±526.80	34.57±8.29	239.00±33.55	46.89±0.84	5.24±0.72
NY50 24mg/kg	18.49±12.16	11.00±1.51	703.19±48.91	1244.91±914.19	1354.95±154.50	5212.89±369.82	60.47±11.43	340.70±51.99*	53.88±9.61	6.06±0.28*
NY50 120mg/kg	15.28±2.52	10.68±0.76	777.84±65.99	858.90±152.11	1997.16±128.83***	7254.66±444.06***##	52.90±5.80	439.98±59.89***	61.91±15.36	7.47±2.10***
NY200 4.8mg/kg	13.91±1.54	12.21±2.06	747.39±55.95	1048.88±264.60	1213.19±381.25	4515.50±1449.96	48.18±13.27	240.32±50.15	50.26±5.69	4.65±0.45
NY200 24mg/kg	19.76±10.66	12.57±2.35	770.36±62.46	1402.81±415.00	1397.01±260.02	5213.59±803.99	62.35±18.67	323.06±67.96*	50.18±8.23	6.06±1.25**
NY200 120mg/kg	14.03±1.13	13.10±2.76	747.32±81.07	895.88±134.40	1735.40±296.57**	5917.56±588.36**	49.50±14.49	378.15±56.82***	61.89±13.46	6.55±0.78**

*p＜0.05, **p＜0.01, ***p＜0.0001 vs control; $^{\#\#}P$＜0.01 indicated as groups of NY50 120mg/kg vs NY200 120mg/kg.

Table S2 Blood-element test and blood coagulation examination of mice after intraperitoneal injection with NaYF4:Eu^{3+} particles

Groups	WBC	RBC	HGB	HCT	MCV	MCHC	RDW		PLT
							SD	CV	
Control	3.26±1.56	8.64±2.93	144.00±8.32	47.51±13.74	57.75±10.50	359.63±228.97	36.73±9.47	24.79±6.52	504.75±224.16
NY50 4.8mg/kg	5.67±2.55	6.89±2.93	133.83±7.33	39.72±11.89	63.55±16.84	396.00±240.16	66.42±38.31	32.33±11.83	501.67±371.63
NY50 24mg/kg	5.25±1.40	7.34±3.85	135.60±9.94	39.80±18.18	60.12±15.41	510.40±491.52	58.48±29.57	32.02±8.66	535.00±413.88
NY50 120mg/kg	9.44±6.20*	6.16±4.03	116.50±12.48**	32.65±18.10	61.30±16.99	655.00±735.36	48.90±25.83	34.03±10.84	675.75±621.55
NY200 4.8mg/kg	5.58±2.52	9.09±0.39	141.17±6.71	48.15±5.20	52.90±4.26	294.83±19.78	34.10±3.20	21.48±0.88	401.00±252.42
NY200 24mg/kg	3.92±0.40	7.25±3.50	143.50±11.90	40.58±12.72	66.45±29.12	403.50±215.95	32.00±1.27	30.03±18.12	486.25±193.58
NY200 120mg/kg	8.37±5.11	7.54±4.04	135.50±15.00	37.88±13.13	64.20±35.12	416.75±236.50	34.58±2.99	32.95±14.29	528.25±313.06

*$p<0.05$, **$p<0.01$, and ***$p<0.0001$ vs control.