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Preparation of giant unilamellar CdTe quantum dot vesicles and their metabolic pathway *in vivo*

WANG XianXiang[1,3*], YANG ZhongKe[2], SAN Zhi[1], PEN Xi[2] & HUANG QianMing[1]

[1]*College of Biology and Science, Sichuan Agricultural University, Ya'an 625014, China*
[2]*College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, China*
[3]*College of Chemistry, Sichuan University, Chengdu 610041, China*

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Glutathione (GSH) capped CdTe quantum dots (QDs) with photoluminescence quantum yields of 61% and the maximum emitting at 601.2 nm were prepared in water phase. Giant unilamellar CdTe quantum dot vesicles (GUVs-CdTe), with diameters larger than 1.5 μm, were obtained using lower-pressure evaporation techniques with soybean lecithin. Compared with other QD liposomes, the entrapment efficiency of GUVs-CdTe for QDs has been significantly improved to 86.3%. After GUVs-CdTe were injected into mice through the tail vein, the fluorescence microscopy of tissue sections showed that GUVs-CdTe could not pass through the blood-brain barrier and air-blood barrier, which were removed mostly by the reticuloendothelial system and were widely distributed in the spleen and the liver. This behavior is the same as the character of the metabolic pathway of giant unilamellar vesicles by intravenous injections in mice.

quantum dots, giant unilamellar quantum dot vesicles, metabolic pathway

1 Introduction

Nanotechnology is a significant innovation following the steam innovation, electricity-age and information technology revolution, which could change human life [1]. Functional nano-materials with special structures are expected to bring more advantages to human life. However, with the industrialization of nanotechnology, the negative effects of these tiny particles have been noticed. Therefore, assessing their security is a significant prerequisite for industrialization. Previous reviews indicated that carbon nanotubes would produce lung damage and their toxicity was much stronger than the carbon black [2]. Nano-gold widely used in the immunoassay and molecular recognition has different levels of toxicological effects for organisms [3], and the toxicity of various forms of nano-crystalline TiO_2 to the lung was significantly different [4], which has been confirmed. Quantum dots (QDs) as a new inorganic material have offered such significant advantages as optical labels with high fluorescence quantum yields, stability against photobleaching, and narrow-symmetrical emission spectrum, which has potential value in cell imaging, tumor markers, ion detection and bio-molecular interaction research [5–7]. QDs are special photoluminescence semiconductors, which are composed of such periodic groups of IIB-IVA as CdSe, CdTe, and ZnSe, or IIIA-VA as InAs and InP, and their diameter is less than or close to the exciton Bohr radius. *In vivo* labeling, heavy metal particles are readily released from QDs, resulting in damage to the different organizations in the body. By means of the QD surface modification, such as surface modification layer of SiO_2 [10] and polyethylene glycol (PEG) [11], they could prevent the release of heavy metal ions to enhance biological compatibility. The liposome is a common carrier transport of drugs in vivo due to its excellent bio-compatibility, which is composed of

*Corresponding author (email: xianxiangwang@hotmail.com)

an orientation double phospholipid molecule in the aqueous phase, and its diameter is from several nanometers to several microns. In terms of the diameter, liposomes are classified as small unilamellar vesicles (SUVs) and giant unilamellar vesicles (GUVs). SUVs are small and uniform vesicles with the minimum diameter of 20 nm or so. The encapsulation efficiency for drugs was only 0.1%–1% and they were unevenly distributed inside and outside the membrane. Therefore, SUVs as drug carriers have limitations. However, the GUVs' diameter is larger than 100 nm and they have a higher rate of encapsulation for water-soluble drugs. They have a distinct advantage as drug carriers [12]. Our previous study [13] found that the hemolysis rate of CdX (X=Se, Te, Te/ZnS) QDs modified with lecithin and cholesterol was less than 5%, which satisfied the requirements of biomedical materials. QDs-liposomes are significantly enriched in brain tissue through the blood-brain barrier in mice. However, the metabolism is different following liposomes with different sizes *in vivo*. For example, the liver allows liposomes with a diameter about 200 nm to pass through, so small liposomes could reach the liver parenchymal cells, and large liposomes are limited in the intravascular space as capillary wall barriers, and uptake by blood mononuclear cells, the liver, spleen, bone marrow and other fixed tissue cells [12]. The toxic effects of QDs were confirmed by many studies concerning their types, sizes and surface modification groups [14]. The liver and the spleen are enriched organs of QDs after injecting into mice from the tail vein [15]. Whether there is a significant difference between the metabolism of QDs-liposomes with different sizes *in vivo* remains a question. On the basis of our previous work [13, 16], we prepared giant unilamellar CdTe QD (GUVs-CdTe) vesicles, and studied the metabolic pathways in mice using fluorescence microscopy, in order to provide a scientific basis for GUVs-CdTe applications.

2 Experimental

2.1 Reagents and instruments

$CdCl_5 \cdot 5H_2O$, mercaptoacetic acid, $NaBH_4$, tellurium powder and other chemical reagents were analytical grade, which were purchased from Chengdu Kelong Chemical Reagent Co., Ltd. Reduced glutathione (GSH) was from Aldrich-sigma Co. Kunming mice were supplied by the Animal Experimental Center of Sichuan Agricultural University.

UV-2000 UV-visible spectrophotometer (Unico), Varioskan full wavelength fluorescence/colorimetric scanner reading instrument (Thermo Electron Co.,), high-speed refrigerated centrifuge (Thermo Electron Co.,), BX51 fluorescence microscope (Olympus), and RE-52AA rotary evaporator (Shanghai Ya-rong biochemical Instru.) were used. The water used is ultra-pure water prepared using the Milli-Q system.

2.2 Preparation of giant unilamellar CdTe QDs

Red CdTe QDs were synthesized in water phase with the assistance of glutathione (GSH) as the stabilizer according to the method developed by Ying *et al*. [17] with slight adjustment. 0.0700 g of tellurium powder and 0.0500 g of $NaBH_4$ were loaded in a 20 mL two-necked flask. The air in the system was pumped off and replaced with N_2. 3 mL of cooled boiled double-distilled water was added through a syringe. The reaction mixture was heated at 80 °C for 10 min under N_2 flow to obtain a deep claret clear solution. The NaHTe solution obtained was stored for later use at room temperature, still under the protection of N_2. 100 mL of 1.0 mmol/L of Cd^{2+} solution and 0.1 mmol of GSH were mixed, and the pH of the solution was adjusted to 10.0 by dropwise addition of 1.0 mol/L NaOH solution with stirring. The solution was placed in a three-necked flask and was deaerated. Under stirring, 300 μL of freshly prepared NaHTe solution was added at room temperature. The reaction mixture was heated to reflux temperature 100 °C under N_2 protection for 80 min. The CdTe-GSH QD solution obtained was concentrated to 10 mL by the rotary evaporator and transferred to 10 mL cold ethanol, and subsequently centrifuged at 10000 r min^{-1}, 4 °C. CdTe-GSH at different concentrations could be obtained using the yellow solid powder.

GUVs-CdTe was synthesized according to the previously reported [13, 18] method with minor modification. In a 100 mL round bottom flask, 30 μL 0.1 mol/L soybean lecithin, 1500 μL chloroform and 300 μL of methanol were mixed. After mixing, 10 mL of the phosphate buffer solution (50 mM, pH 7.4) was slowly dropped along the flask. It was placed for 10 min, 5 mL of 1 mM CdTe-GSH was added, and the organic solvent was slowly evaporated by a rotary evaporator under decompression at 40 °C. About 10 mL milk yellow solution of GUVs-CdTe was obtained, and stored in the refrigerator at 4 °C.

2.3 Determination of the entrapment efficiency

The encapsulation efficiency of liposomes was determined by the ratio of relative fluorescence peak areas. 100 μL of GUVs-CdTe suspension was mixed with 95% ethanol in a 10 mL flask, and 500 μL suspension was placed into the EP tube and centrifuged for 30 min at 4 °C, 12000 r min^{-1}. The integral area S_1 is supernatant solution fluorescence spectra with excitation at 370 nm, and the integral area S_2 is the fluorescence spectroscopy of CdTe-GSH with the same concentration measured under the same conditions. The entrapment efficiency was calculated according to Formula 1:

$$E\% = \frac{S_2 - S_1}{S_2} \times 100\% \quad (1)$$

2.4 Metabolic pathway *in vivo*

After the mice was raised about 2 weeks in the experimental environment, health bodies about 20 g were selected as experimental subjects. According to the mouse weight, the mice were dosed with 0.2 mmol/10 g of GUVs-CdTe through intravenous injection. They were killed after 12 h and dissected to make paraffin sections of the heart, liver, spleen, lung and kidney, and the distribution of QDs in the tissues was observed by a fluorescence microscope.

3 Results and discussion

3.1 Formation of giant unilamellar QD vesicles

Giant unilamellar vesicles could improve the encapsulation efficiency of encapsulated objects, which could be directly observed by optical microscope. Thus, they have wide applications in biological and pharmaceutical research models [19]. Generally, liposomes are obtained by vacuum evaporation method. However, this method requires at least more than 30 min rotation to obtain liposomes with a diameter lager than 1 μm, which often engenders protein denaturation in the water phase and organic phase. Therefore, preparation of giant vesicles is significant for developing effective method. A new route was developed utilizing the excellent optical properties of semiconductor fluorescent dyes to study GUVs' properties in animal bodies. Figure 1 shows the formation of giant unilamellar QDs. Initially, an ordered monolayer of phospholipids was formed at the interface between the water phase and organic phase (Figure 1(a)). In this interfacial region, the polar head groups of phospholipid were located in the aqueous phase, whereas the fatty acid chains appeared in the organic layer. When the organic phase was evaporated under reduced pressure, these ordered structures were ruptured into fragments and forced into the aqueous phase. Fragments were transported by air bubbles because of the surface effect in the edge of the air bubble solution (Figure 1(b)). Phospholipid bilayer fragments were subsequently formed from micelles in the bubble edge (Figure 1(c)), and with the progress of evaporation, unilamellar vesicles containing water-soluble CdTe-GSH (Figure 1(d)) were yielded. According to Formula (1), the encapsulation efficiency of the QDs may increase to 86.3%. The encapsulation efficiency has been significantly improved compared with the previous reports [13]. This demonstrated that giant unilamellar QDs vesicles enhanced the encapsulation efficiency of the objects.

3.2 Spectra and morphology of giant unilamellar QDs

The main synthetic routes of quantum dots are frequently divided into high-temperature decomposition of organic metals and water phase synthesis. Although the QDs' size is uniform, the crystal grows perfectly and possesses outstanding optical properties obtained by high-temperature decomposition of organometallic, but the water solubility and biocompatibility are very poor because the surface is the hydrophobic organic carbon chain. Through ligand exchange, it could be exclusively used in biology. By contrast, the method with water phase synthesis may directly provide satisfactory water solubility although the crystal is not improved and the half-peak width is wider. This is a more green approach. Thiol-containing compounds as ligands were used in water phase synthesis, such as mercaptocarboxylic acid, cysteine and glutathione [20]. In particular, GSH capped QDs synthesized in aqueous phase possess high quantum yields, satisfactory water solubility and biocompatibility. With the surface containing amino and carboxyl functional groups, they are readily modified for fluorescent signals *in vivo*. Figure 2 shows the UV-visible absorption peak of the prepared CdTe-GSH at 580 nm. According to Formula (2), its diameter is 3.50 nm. At excitation of 380 nm, the emission peak is at 601.2 nm, and the quantum yield is 61% with rhodamine 6G as the reference. The emission peak was red shifted to 611.5 nm after the synthesis of GUVs-CdTe, indicating the diameter became larger. The quantum yields decreased to 46%. Figure 3 shows the morphology of GUVs-CdTe under the microscope. The giant unilamellar QDs had a uniform size and

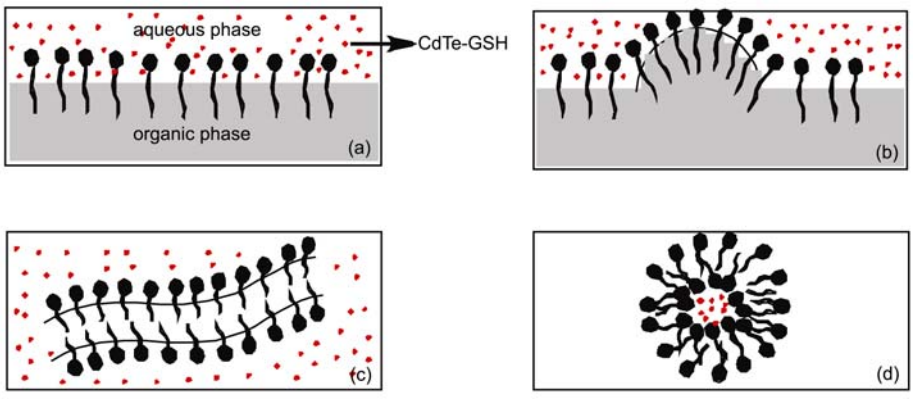

Figure 1 Diagram of the suggested formation mechanism for GUVs.

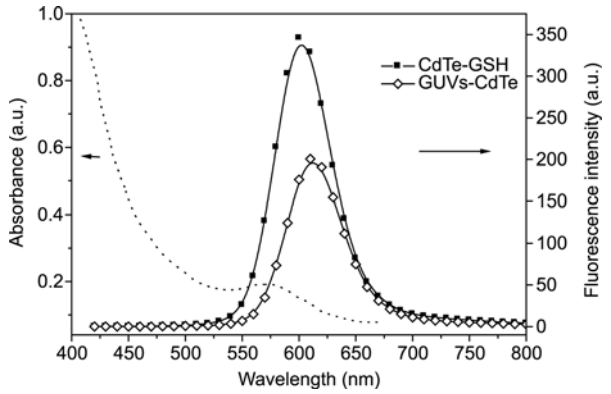

Figure 2 UV-visible spectra (dashed line) and fluorescence spectra of CdTe-GSH QDs with $\lambda_{ex} = 380$ nm excitation.

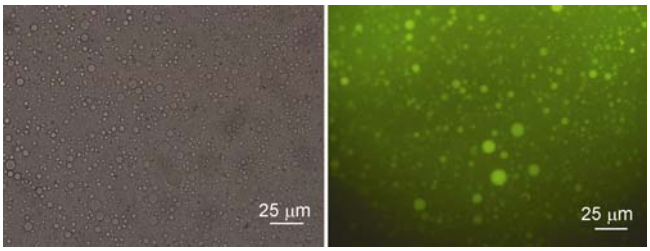

Figure 3 Micrographs of GUVs-CdTe under visible (a) and fluorescence (b) conditions.

the size was about 1.5 μm (statistically 83%), indicating this route could provide a method for the preparation of giant unilamellar QDs.

$$D = (9.8127\times10^{-7})\lambda^3 - (1.7147\times10^{-3})\lambda^2 + (1.0064)\lambda - (194.84) \quad (2)[21]$$

3.3 Metabolism

Previous studies showed that the distribution of liposomes *in vivo* was affected by different ways of administration. One way was to eliminate the liposomes by reticuloendothelial systems (RES) through the vein, in which they were mainly cleared by the liver followed by the spleen and bone marrow. The patterns of clearing liposomes in the blood were decided by the dose, size and composition of the liposome. Larger liposomes have great affinity with RES and could be rapidly cleared. Reports indicated that 80% of GUVs was removed by the liver stellate cells, 4.5% by the spleen and 2% by the bone marrow [12]. Mice were injected with GUVs-CdTe using the previous method and the slicing was observed under a fluorescent microscope (Figure 4). There was no fluorescent signals of QDs-liposomes in the brain and lung slices (showing red fluorescence at excitation), which demonstrated that GUVs-CdTe could not pass through the blood-brain barrier and air-blood barrier. However, there were large gathering areas of red QDs in the heart, indicating GUVs-CdTe was limited in the intravascular space and could exist longer in the blood. A mass of red diffuse QDs were observed in the spleen and liver slices, which was the characteristics of metabolic pathway of giant unilamellar vesicles removed by RES in the liver and spleen, and they were enriched in those tissues after being injected in mice through the tail vein. In the view of pharmacokinetics, substances with a diameter smaller than 400 nm in the blood are easy to be discharged out of the body through the kidney, and do not affect their passing through the liver. Because the size of prepared QDs-liposomes was uneven, a part of the small particles reached the kidney and uptake by the liver. Therefore, QDs were apparently enriched in the liver after intravenous injection in mice. If CdTe-GSH QDs

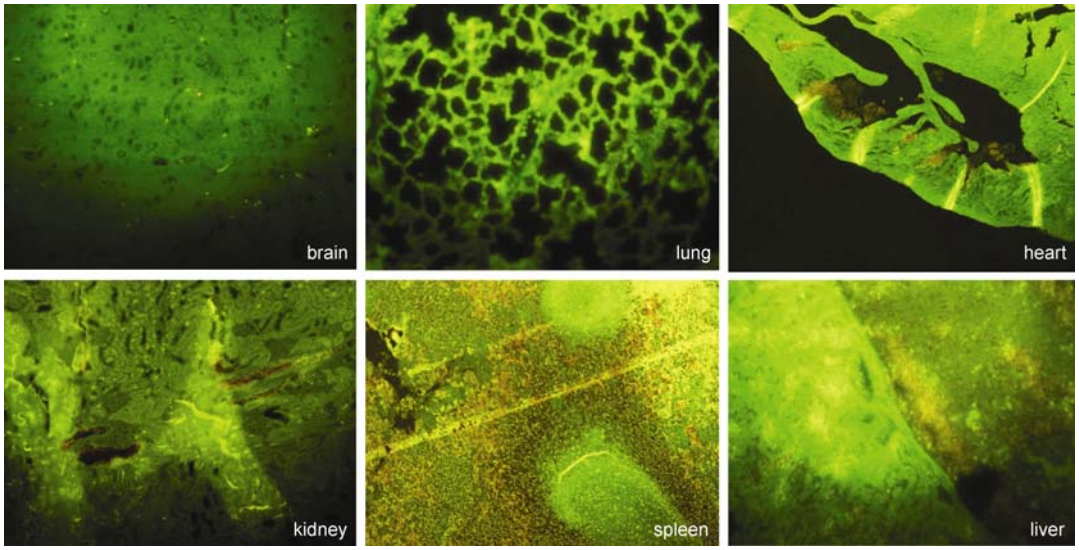

Figure 4 Typical tissue section images of the brain, lung, heart, kidney, spleen and liver under a fluorescence microscope after injecting GUVs-CdTe through the tail vein.

were directly injected through the vein, the apparent aggregation groups of QDs appeared in the liver slice, which intensified the biological toxicity of QDs and rapidly killed the mice (Figure 5). These results were consistent with preliminary hemolysis test [13] and other reports [9]. QDs-liposomes are an effective method to solve the toxicity problem of QDs and ensure security in applications to control the aggregation morphology produced by the QDs with proteins *in vivo* and reduce the formation of aggregation in the liver.

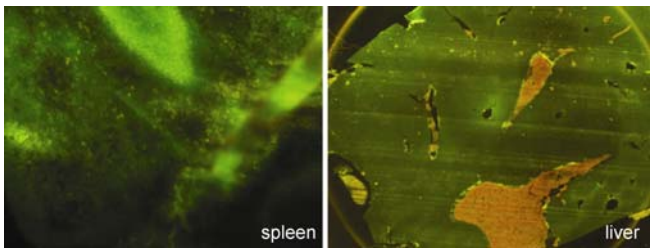

Figure 5 Fluorescence microscope images of the spleen and liver sections after injecting CdTe QDs through the tail vain.

4 Conclusions

QDs as an excellent inorganic fluorescent probe have potential applications in the field of biology, but their toxicity limits large-scale applications. The toxicity decreased after they have been made into QDs-liposomes, satisfying the requirements of their applications as biological materials. There was a notable difference between QDs-liposomes with different sizes in the distribution and metabolism in vivo. The experiments confirmed that giant unilamellar QD vesicles could improve the encapsulation efficiency (86.3%) and GUVs-CdTe widely spread in the spleen and liver and was mainly cleared by the RES. These behaviors were in agreement with the characteristics of metabolic pathway of giant unilamellar vesicles injected in mice through the tail vein. The fluorescence spectrometry is significant in the study of pharmacokinetics [22, 23]. Giant unilamellar vesicle QDs reduced the toxicity of QDs as well as increased the encapsulation of drugs. This research provided scientific data for pharmacokinetic studies *in vivo*.

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