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Quantum Dots Biodistribution in Tissue Organs of Healthy Male and Female Mice

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Abstract: Quantum Dots (QDs) are autoflorescence semiconductor nanocrystals that can be used for *in vivo* biomedical imaging. However, we know a little about their *in vivo* distribution in tissue organs and health consequences. The aim of this study was to detect QDs biodistribution in different organs from healthy female and male mice after single intravenous injection at the dose of 2.98 pmol CdSe/CDs/ZnS QDs/mouse for up to 14 day in female and 8 h in male mice. Laser scanning confocal microscope and/or florescence light microscopy was used to detect QDs in different samples. The results revealed that most of QDs were highly accumulated in spleen, liver, lung of treated mice; however, small amount of QDs was detected in kidney. There is no QDs were observed in other organs such as heart of female mice and brain of male mice of treated group. We also didn't find QDs in all samples prepared from control group and blood sample of treated mice at different time points. Effective and rapid (1 h) detection of tissue organs and blood samples using fluorescent imaging of quantum dots was demonstrated. This work was done using a very low dose (2.98 pmol/mouse) of injected QDs.

Key words: Quantum dots (QDs), biodistribution, organs, mice

INTRODUCTION

Quantum Dots (QDs) are fluorescent inorganic nanometer sized crystals with remarkable unique optical and electronic properties. They are composed of a fluorescent core of semiconductor heavy metals. In order to limit their potentially toxic effects due to the release of Cd^{2+} or Se^{2-} ions in case of oxidation of the core by ultraviolet light and to passivate the defects on its surface, this core is encapsulated in a shell of organic material, polymeric or

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lipid-based layers then coat the shell. The QDs are emerging as a new class of luminescence probes for biology and medicine research applications. The QDs possess unique optical tunability, such as size-tunable emission wavelength, superior signal brightness, resistance to photobleaching and broad absorption spectra for simultaneous excitation of multiple fluorescence colors using a single excitation source (Michalet *et al*., 2005; Medintz *et al*., 2005; Robe *et al*., 2008).

Several studies have shown QDs may be systemically distributed and may accumulate in organs and tissues. Absorption, Distribution, Metabolism and Excretion (ADME) characteristics are, not surprisingly, highly variable for QDs because of the wide variation in QD physicochemical properties. The QD size, charge, concentration, stability and outer coating bioactivity each contribute to not only the potential toxicity of a given QD but also to their ADME characteristics. Physicochemical properties in conjunction with environmental factors and QD stability (oxidative and photolytic lability) together are a paradigm in which ADME characteristics of QDs can be highly variable and difficult to predict (Hardman, 2006). QDs have been already applied to visualize SLN in gastrointestinal tract (Soltesz *et al*., 2006), pleural space (Parungo *et al*., 2005a), lung area (Soltesz *et al*., 2005), oesophageal area (Parungo *et al*., 2005b), skin (Tanaka *et al*., 2006) and axilla (Kim *et al*., 2004) using infrared light. Recently, red and infra-red emitting QDs were imaged in inguinal and axillary lymph nodes in tumor bearing mice already 3 min after local injection (Ballou *et al*., 2007).

Currently, only few detailed works on QDs biodistribution in rodents have been realized (Fischer *et al*., 2006; Gopee *et al*., 2007; Choi *et al*., 2007; Robe *et al*., 2008). The QDs toxicity study is ultimately related to the investigation of their biodistribution in pre-clinical models. More importantly, one can visualize the labeling dynamic process of the bioconjugated QDs in the cellular level using appropriate imaging setup such confocal microscopy and multi-photon imaging techniques (Yong *et al*., 2007, 2009).

The main objective of this study is to observe the QDs biodistribution in the different organs of both male and female post-injection via tail veins using fluorescence and confocal microscopy.

MATERIALS AND METHODS

Experiments of this study have been carried out during 2008 and 2009.

Synthesis of QDs

The CdSe/CDs/ZnS QDs were prepared by growing a CDs/ZnS-graded shell on a CdSe core. Briefly, the reaction was carried out with CdSe core QDs corresponding to 0.3 g, using a Cd: Zn: S ratio of 1: 3: 4 (0.5 mmol Cd, 1.5 mmol Zn and 2 mmol S) and 10 mL of oleic acid at 300°C. The CDs initially prefers to grow on the CdSe dots because the CdSe lattice mismatch with CDs is less than that of ZnS. The CDs layer will mediate the growth of the more strained ZnS. The shell growth is uniform and epitaxial and eventually coats the CdSe core. The QDs were separated from the surfactant solution by the addition of ethanol and centrifugation. The reddish QD precipitate could be readily redispersed in various organic solvents (hexane, toluene and chloroform).

Preparation of Lysine Cross-Link Mercaptoundecanoic Acid Coated QDs

Typically, 3 mmol mercaptoundecanoic acid (MUA) was dissolved in 10 mL of chloroform under vigorous stirring. After stirring for 10 to 15 min, 2 mL of concentrated

(~30 mg mL^{-1}) CdSe/CDs/ZnS QD solution was added into this mixture. Approximately 1 min later, 2 mL of ammonium hydroxide was added to the vigorously stirring solution then was stirred overnight at room temperature. The QDs were separated from the surfactant solution by addition of ethanol and centrifugation. The QD precipitate was re-dissolved in 15 mL DMSO for further lysine cross-linking process. The lysine cross-linked MUA QDs were obtained by mixing DMSO quantum dots solution with both DCC (~30 mmol) and lysine (~15 mmol) under vigorously stirring for 2 h. The lysine coated QDs were precipitated from the solution by addition of ethanol and centrifugation. The red-brownish precipitate was redispersed in 10 mL HPLC water and the solution was further filtered using a syringe filter with a nominal pore diameter of 0.45 µm. The lysine coated QDs have relatively good colloidal stability and no precipitation was observed after several months. This stock solution was kept in the refrigerator at 4°C for further use.

Animals

Four-week-old male and female prepubertal kunming mice were purchased from Guangdong Medical Lab. Animal Centre, China. The mice were acclimated for 2 weeks in the animal facilities at the life science school (Shenzhen, University). Mice were kept in 12 h light/dark cycle and had access to food and water *ad libitum*. Animal procedures were performed in compliance with institutional and national guidelines.

Experimental Groups and QDs Administration

In the present study, two types of experiments were carried out: first experiment, a total of 30 male mice and the second experiment, 42 female mice (six weeks old), weighing from 28 to 31 g were used in this study. Two groups of animals, each composed of 15 mice, were used for biodistribution studies in male mice: each mouse of control group injected with 100 µL of saline buffer and treated group injected with 100 µL containing 2.98 pmol QDs/mouse via tail veins. Three time points were use to check the QDs biodistribution in different organs 2, 4 and 8 h for male mice and 1, 2 and 14 day for female mice after the injection. At the end of each time point, 10 male mice (5 each group) and 14 female mice (7 each group) were killed. After sacrifice, blood samples were separately collected from each mouse. All organs of each mouse including spleen, kidney, heart, lung, brain, liver and testis for males and ovaries for female were removed and made soluble for slides preparation.

Microscopic Investigations

All images were obtained by laser scanning confocal microscope (TCS SP2, Leica, Germany) and/or florescence light microscopy. The excitation wavelength used in this study is 488 nm. Both reflection and fluorescence images were obtained for all organs and acquired simultaneously using the two-wavelength band-selected channels. The wavelength band setups for collecting reflection and fluorescence images were 480-500 and 580-620 nm, respectively. Fluorescence Microscope (Olympus IX71, Japan) was used to obtain images too.

RESULTS

QDs Biodistribution in Female Organs

Female mice were injected with QDs, scarified at 1, 7 and 14 day after injection, all organs removed and slides were prepared from different tissue organs. Results showed that QDs

Table 1: QDs biodistribution in different organs of female mice (day as a time point)

	Organs							
Groups (day)	Blood	Spleen	Liver	Lung	Heart	Brain	Ovary	Kidney
Control	-	-	-	-	-	-	-	-
1	-	+ + +	++	++	-	-	-	+
7	-	+ + +	+++	++	-	+	+	+
14	-	+ +	++	+	-	-	-	-

-: No QD has been detected, +: Small amount, ++: Medium amount, +++: High amounts of Qds

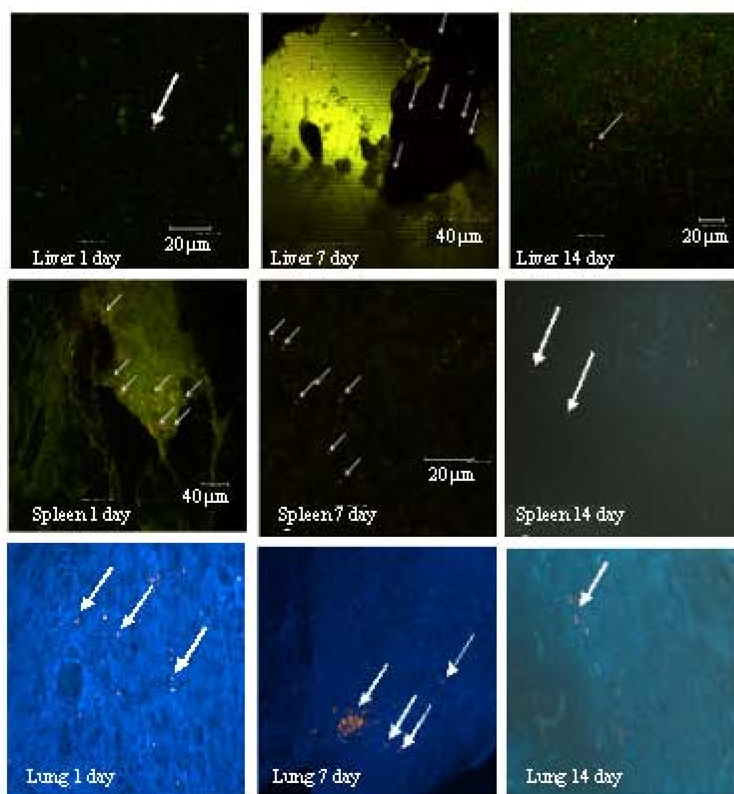

Fig. 1: Examples for confocal and fluorescence microscopy images from tissue organs of female mice 1, 7 and 14 d after single QDs injection via tail veins

have been detected in spleen, liver and lung at different time points. However, QDs have been detected in brain, ovaries and kidney at 7 day after injection. On the other hand, there is no QD has been found in all samples of control group and blood samples from all treated group at different time points (Table 1, Fig. 1).

Qds Biodistribution in Male Organs

In this experiment, as shown in Table 2 and Fig. 2 no QDs have been detected in all samples from control group, brain and blood samples of treated group at all time points. The QDs accumulated in spleen, liver and heart. However, a small amount of QDs has been detected in both testes and kidney from treated group at 8 h post-injection.

Table 2: QDs biodistribution in different organs of male mice (hour as a time point)

	Organs							
Groups (h)	Blood	Spleen	Liver	Lung	Heart	Brain	Testis	Kidney
Control	-	-	-	-	-	-	-	-
2	-	+ + +	+++	++	+	-	-	-
4	-	+ + +	+++	++	-	-	-	-
8	-	+ + +	+++	+++	++	-	+	+

-: No QD has been detected, +: Small amount, ++: Medium amount, +++: High amount of Qds

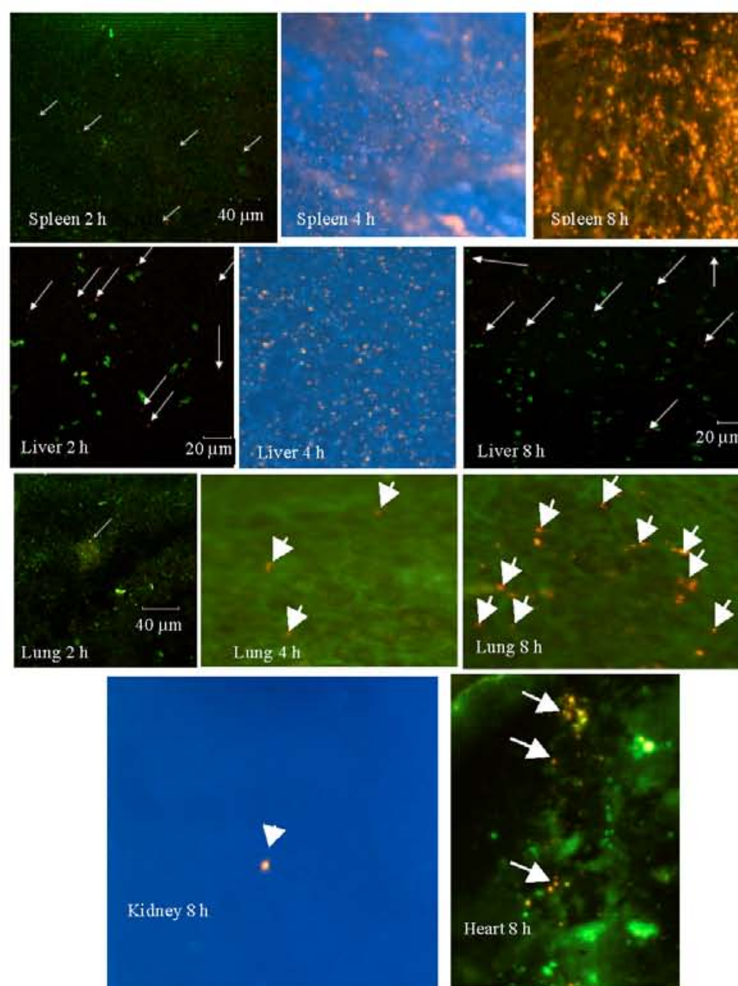

Fig. 2: Examples for confocal and fluorescence microscopy images from tissue organs of male mice 2, 4 and 8 h after single QDs injection via tail veins

DISCUSSION

In the present study, the injected mice were viable and healthy until the end of each experiment. Moreover, no noticeable ill effects in mice injected with QDs. In an *in vivo* study

employing mice, Ballou *et al.* (2004) injected (iv) amphiphilic polyacrylic acid polymer-coated QDs (amp-QDs) and amp-QDs conjugated to PEG-amine groups (mPEG-QDs), at QD concentrations of 20 pmol QD/g animal weight. Necropsy showed no signs of necrosis at the sites of tissue deposition and injected mice were viable for 133 day until the time of necropsy. No obvious sign of QD breakdown *in vivo* was detected by electron microscopy (*in vivo* QD stability was presumed to be due to the amphiphilic polymer coating). In another *in vivo* study, Larson *et al.* (2003), observed no noticeable ill effects in mice injected (iv) with 20 nm and 1 μm solutions of CdSe/ZnS QDs (ill effects was not defined).

In general, QDs distribution depends on their physicochemical properties and in particular, size and coating. Tracer particle size has an important effect on migration time. Particles less than 5 nm diffuse into blood, those between 5 to 10 nm can migrate through nodal tissue and result in false-positive results and those higher than 1000 nm largely remain at the injection site (Parungo *et al.*, 2005a; Tanaka *et al.*, 2006). We used QDs coated with lysine with a size ranged from 20 to 25 nm and the QDs were detected at 2, 4 and 8 h post-injection in male organs and 1, 7 and 14 day in female organs, especially in spleen, liver and lung. It has been reported that QDs with a diameter of 16 nm, since a size range between 15 and 20 nm is optimal to enable the QDs to travel through the lymph channels and be trapped by SLN (Robe *et al.*, 2008). The distribution in healthy mice of 48 pmol intradermally injected QDs with a diameter of 37 nm and PEG coating has been investigated by Gopee *et al.* (2007). The authors did not find any QDs in lymph nodes until 12 h after injection, whereas Robe *et al.* (2008) could detect them already 5 min following the injection. An early detection (3 min) of QDs traveling toward inguinal nodes was observed after intratumoral injection of red emitting QDs at concentrations similar to ours (Ballou *et al.*, 2007). However, this observation was not followed by biodistribution investigations. The QDs fluorescence increases with time to reach a maximum at 60 min after the injection.

Data obtained from the present study showed that most of QDs were highly accumulated in spleen, liver, lung from male and female mice; however, small amount of QDs was detected in kidney and there is no QDs were observed in other tissue organs such as heart of female and brain of male mice. In contrast to our results, the previous QDs biodistribution studies all evidenced the presence of QDs in the liver and also to a minor extent in spleen and kidneys (Fischer *et al.*, 2006; Gopee *et al.*, 2007). *In vivo* studies suggest that, regardless of the specificity of the QD, vertebrate systems tend to recognize QDs as foreign, with elimination of the materials through the primary excretory organs/systems.

CONCLUSION

In this study, we demonstrated that this rapid and selective accumulation of QDs in different tissue organs of female and male. Effective detection of tissue organs and blood samples using fluorescent imaging of quantum dots was demonstrated. This study was done using a very low dose (2.98 pmol/mouse) of injected QDs.

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