SPECT/CT imaging of radiolabeled Quantum dots

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1. Introduction

Quantum dots (QDs) are emerging as a new fluorescent probe for *in vivo* and *in vitro* imaging. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties, such as size-tunable fluorescence emission, improved signal brightness and resistance against photobleaching [1]. The well-defined *in vivo* behavior of QDs is prerequisite information for biomedical application. To identify *in vivo* behavior of QDs, several efforts were done by using *in vivo* fluorescence imaging and inductively coupled plasma atomic emission spectroscopy (ICP-AES) [2]. However, *in vivo* tracking and quantification of QDs by fluorescence imaging is limited by tissue absorption of light and ICP-AES can not be used to measure consecutive and temporal accumulation of QDs in living animals. Scintigraphy is a standard nuclear medicine approach and clinically established to characterize lesions. Radiolabeld QDs will provide quantitative and temporal information of QDs' *in vivo* behavior. Here we evaluated the feasibility of radio-iodination of QDs using Bolton-hunter reagent. The biodistribution of radioiodinated QDs were measured by using gamma counting and *in vivo* distribution of radiolabeled QDs were evaluated by SPECT/CT imaging

2. Methods and Results

2.1 Radiolabeling of quantum dots using radioiodine labeled sulfo-SHPP

Qdot 800 ITK amino polyethylene glycol (PEG) (Invitrogen) are coated with a proprietary polymer and covalently conjugated with 2,000 MW PEG. QDs were radiolabeled with $Na^{125}I$ using the Bolton-Hunter methods [3] with minor modifications. Briefly, radioiodination was performed in sodium phosphate buffer containing 3.7 MBq of $Na^{125}I$. The radioiodine solution was placed in IODO-GEN precoated tube and pre-incubated for 30 min at room temperature. sulfo-succinimidyl-3-(4-hydroxyphenyl)propionate (sulfo-SHPP) dissolved in dimethyl sulfoxide added to reaction vial, then immediately transferred to 25 pmol of QDs. This mixture was incubated for 1 h at room temperature with gentle mixing. Unincorporated radioiodine was removed by centrifugal filtration. QDs were radiolabeled with $Na^{125}I$ by using a water soluble Bolton-hunter reagent (sulfo-SHPP) (Fig. 1). The ^{125}I-HPP-QDs were prepared with a radiolabeling yield of 33.4 ± 2.0%. Our radiolabeling yields (approx. 30%) were in agreement with previous study which has been labeled with Bolton-hunter reagent [4].

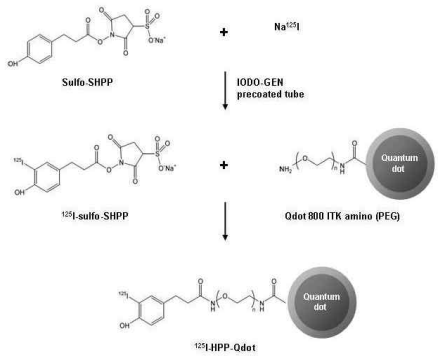

Fig.1. Preparation of ^{125}I-sulfo-SHPP and radiolabeling of the Qdot 800 ITK amino (PEG).

2.2 Biodistribution of Radiolabeled QDs

For evaluating the biodistribution of radiolabeled QDs, QDs labeled with ^{125}I-sulfo-SHPP (1.11 MBq/25 pmole/head, n=4) were administered into the tail vein of the mice. At 2 h and 24 h post-injection, the mice were sacrificed and the blood and various tissues were excised for radioactivity in a well-type gamma counter. The radioactivity of tissues was expressed as the percentage of injected radioactivity dose per gram of tissue (%ID/g). The quantitative distribution of ^{125}I-HPP-QDs is shown in Table 1. The highly accumulated radioactivity in liver (56.3 ± 1.7 %ID/g at 2 h and 48.3 ± 7.6 %ID/g at 24 h) and spleen (61.0 ± 5.0 %ID/g at 2h and 64.2 ± 5.1 %ID/g at 24 h) retained throughout the remainder of the study. However, radioactivity in lung decreased from 76.3 ± 20.8 %ID/g at 2 h to 23.0 ± 10.7 %ID/g at 24 h. It may be caused by blood clearance of radioiodinated QDs. Inoue et al. [5] showed that systemically administered QDs were captured by reticuloendotherial systems (RES) such as liver and spleen. In our experiment, uptake of liver and spleen also showed relatively high accumulation of QDs. In contrast to previous report [2], *ex vivo* gamma counting of isolated tissues showed lung localized radioactivity at 2 h. However, the absorption, distribution, metabolism and excretion characteristics of QDs are highly variable because of the wide variation in

QDs physicochemical properties and environmental conditions. Those factors may contribute to biodistribution of radioiodinated QDs [6].

Table 1. Biodistribution (%ID/g) of radiolabeled QDs in mice

Tissue	^{125}I-HPP-QDs	
	2 h	24 h
Blood	2.8 ± 1.4	0.2 ± 0.0
Heart	1.0 ± 0.3	0.3 ± 0.2
Liver	56.3 ± 1.7	48.3 ± 7.6
Lung	76.3 ± 20.8	23.0 ± 10.7
Spleen	61.0 ± 5.0	64.2 ± 5.1
Kidney	2.8 ± 0.4	0.6 ± 0.1
Stomach	15.6 ± 3.4	0.3 ± 0.3
Small Int [§]	7.0 ± 4.7	0.2 ± 0.0
Large Int [§]	12.0 ± 4.9	0.6 ± 0.2
Thyroid	1.0 ± 0.4	2.9 ± 1.5
Muscle	2.0 ± 2.2	0.2 ± 0.1
Femur	10.6 ± 2.5	6.8 ± 1.7
I. Lymph[§]	1.8 ± 1.1	0.2 ± 0.1
A. Lymph[§]	1.1 ± 0.2	0.2 ± 0.1
Brain	0.1 ± 0.0	0.04 ± 0.01

All the values are given as a percentage of the injected activity per gram of tissue

[†]Data are presented as mean ± SD for 4 animals

[§] Small Int., Small intestine; Large Int., Large intestine; I. Lymph., Inguinal lymph node; A. Lymph., Axillary lymph node.

2.3 SPECT/CT imaging

For the evaluation of *in vivo* biodistribution of radiolabeled QDs, single photon emission computed tomography and computed tomography (SPECT/CT) images were obtained at 2 h, 24 h and 96 h postinjection of ^{125}I-HPP-QDs (3.7 MBq/100 pmole/head, n=2) by using a small animal SPECT/CT system. The SPECT images of radiolabeled QDs were acquired by setting the energy windows of ^{125}I from 24 keV to 46 keV. Dual gamma cameras equipped with mouse general purpose (MGP) collimator of one hole, 1.0 mm diameter (1.2 mm resolution) were used for SPECT images. The CT images were acquired with X-ray voltage of 70 KVp, anode current of 400 μA, an exposure time of 500 milliseconds for each of 360 rotational steps. CT images were reconstructed with COBRA-EXXIM software (version 6.3.39). The SPECT image provides the spatial and temporal distribution of radiolabeled QDs and CT image provides anatomical information. SPECT/CT images revealed that radiolabeled QDs were mainly localized in the liver and spleen of mice both 2 h and 24 h post injection (Fig. 2). The radioactivity of liver maintained until 96 h post injection. Those result consistent with *ex vivo* gamma counting data. In early phase (2 h), radiolabeled QDs was also localized in lung, heart and intestine region. These radioactivities may be caused by blood pool radioactivity and metabolized radioiodine from radiolabeled QDs in *in vivo* environment.

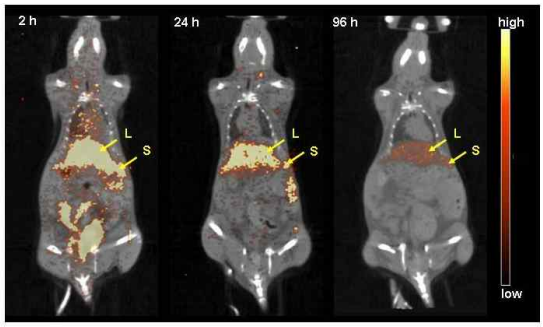

Fig.2. SPECT/CT images of ^{125}I-labeled QDs in normal mice. SPECT/CT images showed high uptake in liver and spleen, moderate uptake in lung and intestine at 2 h. Coronal images was ventral view. L is the liver, S is the spleen.

3. Conclusions

Radiolabeling strategies have offered the highest sensitivity and are mostly quantitative for the evaluation of biodsitribution. Radioiodination method of QDs using sulfo-SHPP could be easily used for the preparation of a radioiodinated QDs. The biodistribution results from gamma counting and SPECT/CT imaging showed similar pattern and accumulation of the QDs in the RES. We evaluated the radioiodination of QDs using ^{125}I-sulfo-SHPP and the quantitative, temporal and spatial biodistribution of radiolabeled QDs. These results suggest that radioiodination method of nanoparticles using Bolton-hunter reagent could be easily applied for the evaluation of nanoparticles in preclinical study and translational research for clinical application.

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