COMMUNICATIONS

Blood Circulation and Tissue Biodistribution of Lipid−Quantum Dot (L-QD) Hybrid Vesicles Intravenously Administered in Mice

Wafa' T. Al-Jamal,[†] Khuloud T. Al-Jamal,[†] Andrew Cakebread,[‡] John M. Halket,[‡] and Kostas Kostarelos*,[†]

Nanomedicine Lab, Centre for Drug Delivery Research, The School of Pharmacy, University of London, London WC1N 1AX, and Department of Forensic Science & Drug Monitoring, Kings College London, London SE1 9NH, United Kingdom. Received February 5, 2009; Revised Manuscript Received June 25, 2009

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The present work describes the pharmacokinetics of recently developed liposome−quantum dot (L-QD) hybrid vesicles in nude mice following systemic administration. Hydrophobic QD were incorporated into different bilayer compositions, and the serum stability of such hybrid vesicles was evaluated using turbidity and carboxyfluorescein release measurements. L-QD hybrid blood profile and organ biodistribution were also determined by elemental (cadmium) analysis. Following intravenous administration, different tissue biodistribution profiles and tissue affinities were observed depending on the L-QD lipid bilayer characteristics. Immediate blood clearance was observed with cationic (DOTAP/DOPE/Chol) hybrid with rapid lung accumulation, while incorporation of PEG at the surface of zwitterionic vesicles dramatically prolonged their blood circulation half-life after systemic administration. Overall, the L-QD hybrid vesicle system is considered a viable platform that allows QD delivery to different tissues through facile modulation of the hybrid vesicle characteristics. In addition, L-QD offers many opportunities for the development of combinatory therapeutic and imaging (theranostic) modalities by incorporating both drug molecules and QD within the different compartments of a single vesicle.

INTRODUCTION

Quantum dots (QD) are being intensively explored as fluorescent probes for long-term and multimodal imaging purposes *in vitro* and *in vivo* (*1*). However, the low hydrophilicity of QD is considered a major obstacle that impedes their widespread use in biology. Many strategies have been developed to improve the poor hydrophilicity, therefore biocompatibility, of QD, including attachment of hydrophilic moieties to their surface (*2, 3*), encapsulation within amphiphilic copolymer micelles (*4*), phospholipid micelles (*5*), silica polymers (*6*), emulsions (*7*), and polystyrene particles (*8*). These different coating strategies minimize QD nanocrystal aggregation (*9, 10*), protect the QD fluorescence character (*11*), and have also been reported to reduce their cytotoxicity (*2*). We have recently explored an alternative strategy by the self-assembly of lipids and quantum dots (L-QD) into hybrid bilayers, leading to the formation of nanoscale vesicles (*12*). The embedding of QD into the bilayers rendered nonfunctionalized QD compatible with the aqueous environment and enhanced the QD photostability, and most importantly, the L-QD vesicles labeled tumor cells efficiently *in vitro* and *in vivo*. L-QD hybrid vesicles are considered relevant for clinical use since their vesicular morphology allows loading of their internal aqueous phase with therapeutic molecules, in this way constituting a potential combinatory (theranostic) system for the simultaneous delivery of therapeutic and diagnostic agents.

In this work, we explored the use of various types of L-QD hybrid vesicles as delivery systems for *in vivo* systemic circulation applications. Their stability and release profiles in mouse serum were evaluated by turbidimetry and by using vesicle-encapsulated carboxyfluorescein. Further, the blood circulation and tissue biodistribution profiles of the different L-QD hybrid vesicles following intravenous (i.v.) administration in mice were determined, in comparison to the more widely used (and commercially available) poly(ethylene glycol) (PEG)-coated functionalized QD.

RESULTS

L-QD hybrids were prepared following the thin lipid film hydration protocol as previously described (*12*). Briefly, 5.6×10^{14} p/mL CdSe/ZnS QD (960 pmol/mL) were mixed with 8 μmol phospholipid molecules in chloroform, and vesicles were formed following film hydration and sonication, resulting in L-QD hybrid vesicles of 80−100 nm average diameter (Figure 1). The surface charge characteristics of the L-QD hybrid vesicles were in accordance with the characteristics of the lipid molecules used to form the bilayers. L-QD hybrids containing cationic (DOTAP[1]) and PEGylated (DSPE-PEG_{2000}) lipids exhibited surface charges of +47.5 mV and −20 mV, respectively (Figure 1). Also, the characteristics of the bilayers were dependent on the lipid composition selected, namely, fusogenic,

* To whom correspondence should be addressed. Phone: ++44-207-753-5861, Fax: + +44-207-753-5942, E-mail: kostas.kostarelos@pharmacy.ac.uk.

[†] University of London.

[‡] Kings College London.

[1]Abbreviations: DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DSPC, distearoylphosphatidylcholine; Chol, cholesterol; DSPE-PEG_{2000}, distearoylphosphatidylethanolamine-poly(ethylene glycol) of molecular weight 2000; CdSe, cadmium selenide; ZnS, zinc sulfide; ICP-MS, inductively coupled plasma mass spectroscopy.

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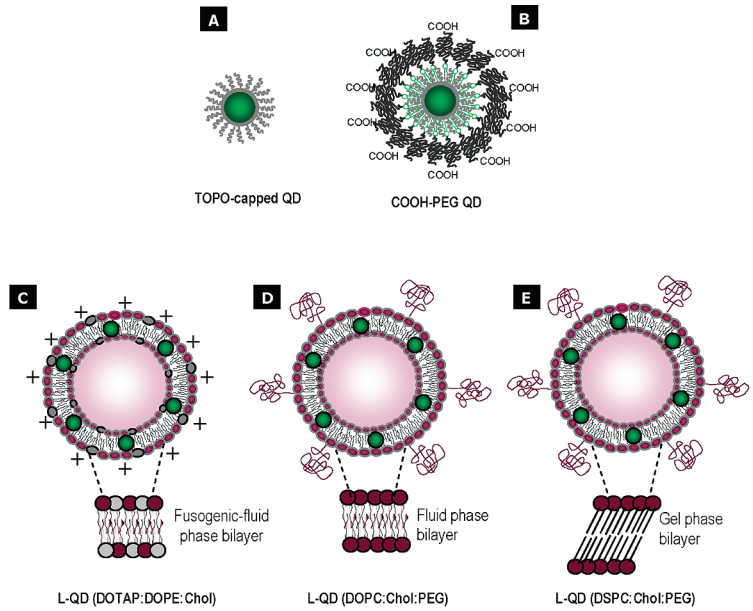

QD Nanoparticle Type	Hydrodynamic Diameter (nm) ±S.D [a]	Polydispersity Index ±S.D [a]	Zeta potential (mV) ±S.D [a]
TOPO-capped QD	2.2 [b]	n/a	n/a
COOH-PEG QD	39.0 (0.00827)	0.167 (0.013)	-7.63 (2.02)
DOTAP:DOPE:Chol (2:1:1.5)	88.8 (0.865)	0.269 (0.005)	+47.5 (1.51)
DOPC:Chol:DSPE-PEG$_{2000}$ (1.8:1:0.2)	104 (0.727)	0.220 (0.010)	-24.0 (1.51)
DSPC:Chol:DSPE-PEG$_{2000}$ (1.8:1:0.2)	111 (0.959)	0.220 (0.001)	-19.5 (0.282)

[a] Mean ±standard deviation; n=3, [b] QD core size according to the manufacturer , n/a: not applicable

Figure 1. A schematic diagram showing a simplified structure of (a) TOPO-capped QD, (b) COOH-PEG QD, and the L-QD hybrids consisting of (c) DOTAP/DOPC/Chol (2:1:1.5), (d) DOPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2) and (e) DSPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2) (diagrams are not drawn to scale). The size, polydispersity index, and zeta potential of COOH-PEG QD and L-QD hybrids used in this study were obtained by dynamic light scattering using Nanosizer ZS.

fluid-phase using DOTAP/DOPE/Chol; fluid-phase using DOPC/Chol/DSPE-PEG$_{2000}$; and gel-phase using DSPC/Chol/DSPE-PEG$_{2000}$.

To evaluate the stability of L-QD hybrid vesicles in physiological conditions, 200 μL (1.6 μmol) of plain liposomes or L-QD vesicles in 5% dextrose were incubated with 1 mL 50% (v/v) CD-1 mouse serum at 37 °C to mimic the *in vivo* environment (approximately half of the volume of complete blood is serum (*13*)). The effect of serum on plain liposomes and L-QD vesicles was monitored by measuring the changes in the turbidity and mean vesicle diameter. Samples were incubated at 37 °C and analyzed at 2, 5, 10, 30, and 60 min using a Beckman DU 640 spectrophotometer (USA). Turbidity was measured at 400 nm with the corresponding amount of serum alone used as a reference. The mean vesicle diameter was measured by dynamic light scattering using the NanoZS (Malvern, UK). All samples were incubated for 60 min in serum.

Figure 2 depicts the stability of L-QD hybrid vesicles incubated in 50% (v/v) CD-1 mouse serum at 37 °C over time. Turbidity increased sharply for both liposomes (diamonds Figure 2a) and L-QD vesicles (diamonds Figure 2b) of the cationic DOTAP/DOPE/Chol lipid composition and was found to be constant over 60 min and 24 h (data not shown), similar to previous observations with cationic systems (*13*). Cationic vesicle aggregation was confirmed by light scattering, as evidenced by increases in the mean diameter from 100 nm to more than 300 nm after 60 min serum incubation (black bars, Figure 2c,d). To determine if changes in cationic vesicle size were due to incubation at 37 °C, all vesicle systems were also incubated in 5% dextrose under the same conditions; however, no changes in size were observed (white bars, Figure 2c,d). PEGylated (sterically stabilized) liposomes and L-QD vesicles (those containing DSPE-PEG$_{2000}$) showed no changes in turbidity (Figure 2a,b) and size (Figure 2c,d) due to the presence of PEG polymer on the vesicle surface (Figure 1) that reduces interactions with serum proteins (*14*). On the basis of these results, only sterically stabilized formulations were used further to determine the release profile of the vesicles.

In order to evaluate the integrity of L-QD vesicles in serum, a high concentration (0.2 M) of the aqueous marker carboxyfluorescein (CF) was encapsulated in the vesicles and free CF was removed by size exclusion chromatography. Release from plain liposomes and L-QD hybrid vesicles were studied by monitoring the increase in the CF fluorescence signal. Rapid release was observed from the L-QD (DOPC/Chol/DSPE-PEG$_{2000}$) vesicles that released 30% of encapsulated CF after 5 min and 50−70% between 0.5 and 4 h (□, Figure 3a). Similar results were obtained for the equivalent liposome system (■, Figure 3a), suggesting that the presence of QD in this vesicle bilayer did not dramatically change the bilayer characteristics that could lead to increased CF leakage.

Modification of the vesicle bilayer by substitution of the fluid-phase DOPC bilayer (Figure 1d) with the "rigid" DSPC (high phase transition lipid in gel phase at 37 °C) (Figure 1d,e),

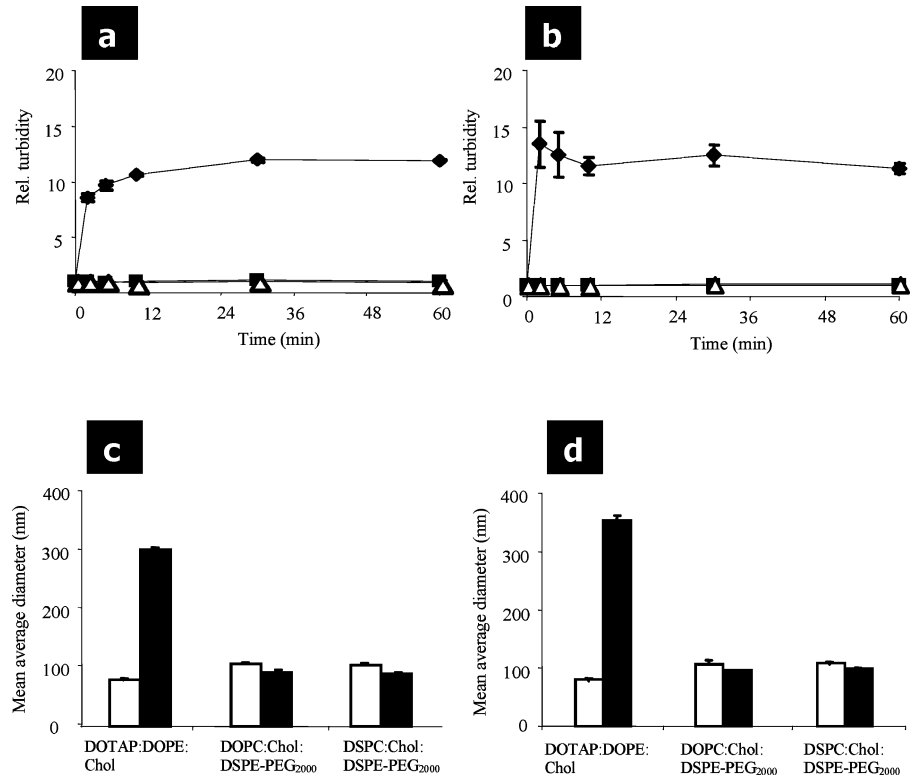

Figure 2. Stability of L-QD vesicles incubated in 50% mouse serum. Relative turbidity at 400 nm of (a) liposomes and (b) L-QD hybrid vesicles consisting of (◆) DOTAP/DOPE/Chol (2:1:1.5); (■) DOPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2); and (Δ) DSPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2); incubated for 2, 5, 10, 30, and 60 min in 50% mouse serum at 37 °C. Mean diameter (nm) of the (c) liposomes and (d) L-QD hybrids after 60 min incubation in 5% dextrose (white bars) and 50% mouse serum (black bars) at 37 °C. Data represent the mean ± S.D.(n = 3).

retarded the CF release from liposomes by a factor of 2, as described previously (*15*). The percentage of CF released from "rigid" [DSPC/Chol/DSPE-PEG$_{2000}$] liposome vesicles was 30−40% after 9 h incubation in serum (▲, Figure 3b). The presence of QD in the DSPC/Chol/DSPE-PEG$_{2000}$ bilayer almost completely prevented CF release up to 72 h (white bars, Figure 3c), indicating the dramatically improved stability of these PEGylated L-QD hybrids at physiological conditions. More biophysical studies are warranted to further elucidate the effect of QD embedding within the lipid bilayer and the interactions at the molecular level between the lipids and the hydrophobic QD self-assembling into vesicles.

We have previously reported that L-QD made of cationic and fusogenic lipid bilayers were capable of effectively labeling cancer cells *in vivo* following intratumoral administration (*12*). To investigate the behavior of L-QD hybrid vesicles in a living animal further, we studied their blood clearance and tissue biodistribution following intravenous (tail vein) administration compared to PEGylated, functionalized QD (COOH-PEG-QD). All pharmacokinetic and biodistribution data in this study were generated by detecting the amount of Cd atoms in the animal tissues using ICP-MS (Supporting Information for details).

Cationic L-QD hybrid vesicles (DOTAP/DOPE/Chol) exhibited rapid clearance from blood circulation with 20% and 1% of the injected dose (% ID) circulating in the blood after 2 and 10 min, respectively (Figure 4a, circles). The rapid clearance is due to transient lung accumulation, where more than 70% of ID was found after 10 min compared to 25% in the liver and spleen (Figure 4b, black bars). At 24 h, redistribution of the cationic L-QD led to their localization mainly in the liver (90% ID) and to a lesser extent in the spleen (5% ID), leaving only 4% in the lung (Figure 4c, black bars). Transient lung accumulation after systemic administration has been described previously for a variety of positively charged nanoparticles (*16*) and is thought to be due to the cationic L-QD interacting with the lung capillaries—the first capillary bed encountered following tail vein administration (*17*−*21*). At later time points, adsorption of negatively charged plasma proteins on the surface of the cationic vesicles hinders the nonspecific interaction with the pulmonary endothelium, leading to their redistribution to liver and spleen (at 24 h) (*17*−*22*). Similar observations have been previously reported for cationic liposome/plasmid DNA complexes (lipoplexes) (*23, 24*).

The blood circulation and tissue biodistribution of sterically stabilized L-QD hybrid vesicles containing 10 mol % of DSPE-PEG$_{2000}$ (*25*) was studied next. PEGylated L-QD (DOPC:Chol: DSPE-PEG$_{2000}$) vesicles exhibited 5−10-fold increase in blood circulation during the first 60 min (Figure 4a, empty diamonds) compared to cationic L-QD (Figure 4a, circles). However, only 10% ID could be detected in the blood after 10 min due to almost complete (>90%) and rapid liver uptake (Figure 4b, white bars). Substitution of DOPC for DSPC to form "rigid" L-QD vesicles (DSPC/Chol/DSPE-PEG$_{2000}$) exhibited up to 30-fold increase in blood circulation during the first 60 min (Figure 4a, squares) compared to DOPC/Chol/DSPE-PEG$_{2000}$ and cationic (DOTAP/DOPE/Chol) L-QD vesicles, with lower RES uptake (Figure 4b, gray bars) as has been previously reported for liposomes (*26*). However, the retention in the blood compartment of these "rigid" L-QD vesicles could not be maintained over time, with only 3% ID detected in the blood after 240 min (Figure 4a, squares).

The pharmacokinetic behavior of L-QD hybrid vesicles was also compared to that of commercially available hydrophilic QD (at 192 pmol—same concentration of QD used throughout) using carboxyl-functionalized poly(ethylene glycol) coated QD (COOH-PEG QD) (Evident Technologies, USA). The hydrodynamic diameter and surface charge of this material were found to be 39 nm and −7.6 mV (Figure 1) injected without further modification via the mouse tail vein. COOH-PEG QD showed prolonged blood circulation (Figure 4a, filled diamonds) as 90%

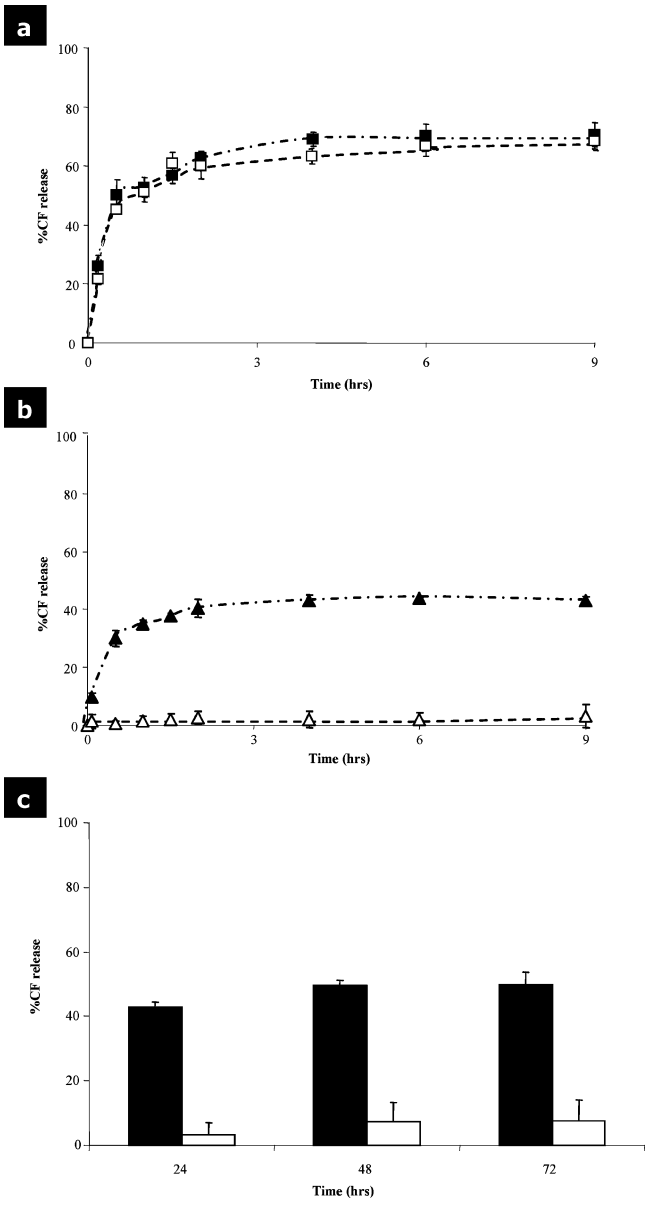

Figure 3. CF release profile from liposomes and L-QD hybrid vesicles incubated in 50% mouse serum. Percentage of CF release from (a) plain (■) and L-QD DOPC/Chol/DSPE-PEG_{2000} (1.8:1:0.2) (□) vesicles; (b) plain (▲) and L-QD DSPC/Chol/DSPE-PEG_{2000} (1.8:1:0.2) (Δ) vesicles incubated in 50% mouse serum at 37 °C for 9 h; and (c) 72 h (plain liposomes, black bars; and L-QD, white bars). Data represent the mean ± SD ($n = 3$).

ID and 20% ID were detected in the blood after 10 min and 24 h (Figure 4c, hatched bars). The tissue biodistribution of COOH-PEG QD was found to be sharply different from that of the L-QD hybrids at the early time point (10 min) with only 4% ID in the liver and spleen. At 24 h postinjection, the COOH-PEG QD concentration in the blood decreased due to accumulation primarily in the liver. No significant accumulation in the kidney, brain, and heart was obtained with either COOH-PEG QD or L-QD hybrids in this study, consistent with previous reports using COOH-PEG QD (*11, 27*).

DISCUSSION

QD have been previously investigated for *in vivo* imaging to visualize tumors, angiogenic endothelium, and lymph nodes in living animals (*1, 11, 28*−*30*). Better understanding of QD structure, surface, and elemental content on their pharmacological behavior *in vivo*, such as their blood circulation and organ biodistribution, is needed. Polymer-coated QD are the most relevant for clinical applications due to their stability *in vivo* and low toxicity (*2, 11*). Moreover, consistent with findings described here, decorating the QD surface with PEG_{2000} can significantly improve their blood circulation ($t_{1/2} = 4-5$ h) (Figure 4a) and reduce their recognition by the liver and spleen (Figure 4b,c) (*1, 11, 29*). We recently described an alternative approach to solubilize organic QD by engineering self-assembled liposome-QD (L-QD) hybrid vesicles that were capable of successful labeling of tumor cells *in vitro* and *in vivo* (*12*). One of the key advantages offered by the L-QD hybrid system is the versatility of potential structural and surface characteristics by selection of different lipid components with minimal manipulation of the QD nanocrystal. Also, they offer the possibility to encapsulate therapeutic agents (e.g., doxorubicin) in their internal aqueous phase for the construction of multimodal (therapeutic and diagnostic) delivery devices. In this study, we explored this versatility by constructing different types of L-QD by rational selection of different molecular (lipid) components (Figure 1) and correlated their composition with their stability *in vitro* and their pharmacokinetic profile *in vivo*.

Our *in vitro* investigations indicated that L-QD hybrids of cationic surface character and a fluid-phase bilayer were not stable in serum and released encapsulated carboxyfluorescein rapidly (Figures 2 and 3). On the other hand, L-QD hybrids consisting of a "rigid", gel-phase lipid bilayer (DSPC) and cholesterol exhibited longer blood circulation (Figure 4a) and slower accumulation in liver (Figure 4b,c) compared to fluid-bilayer or cationic formulations, which is in agreement with earlier observations regarding the effect of liposome compositions on their behavior *in vivo* (*25, 26*). L-QD hybrids with PEG on their surface exhibited higher serum stability (Figure 2), indicating that such L-QD hybrids could offer a platform for systemic multimodal applications (*31*). However, on systemic (i.v.) administration sterically stabilized, PEGylated L-QD showed rapid blood clearance compared to previous reports on similar liposome-alone systems (*25, 26*).

The rapid blood clearance of these hybrids, despite PEG surface coating, can be due to (a) translocation of the hydrophobic QD from the hybrid bilayer to the plasma proteins leading to subsequent uptake by the liver; (b) suboptimal PEGylation of the vesicles containing QD leading to rapid clearance of this vesicle population only (*25*). More work on improving the steric stabilization of L-QD vesicles and their pharmacological profile is warranted by using different lipid molar ratios, PEG of different molecular weights, and optimization of the PEG surface conformation (mushroom or brush) on the L-QD surface (*32*). On the basis of the pharmacokinetic data obtained, L-QD hybrid systems will not be suitable candidates as blood-pool imaging agents due to their rapid clearance from systemic circulation.

Several studies have reported the effect of QD surface coating on QD behavior *in vivo* (*27, 28*). Surface coating determines the overall QD size; therefore, high molecular weight polymers or organic coatings lead to considerable increases in QD diameter that accelerate QD blood clearance and liver entrapment. Such effects have been previously reported by Fischer et al. who observed remarkable differences in the blood profile of 25 nm mercaptoundecanoic acid cross-linked with lysine-QD (LM-QD) ($t_{1/2} = 60$ min), compared to 80 nm bovine serum albumin coated QD (BSA-QD) ($t_{1/2} = 38$ min) (*27*). In addition, QD surface charge was also found to significantly affect QD tissue biodistribution. The cationic LM-QD accumulated significantly in the lung and kidney compared to the zwitterionic BSA-QD that accumulated predominantly (99% ID) in the liver (*27*). More

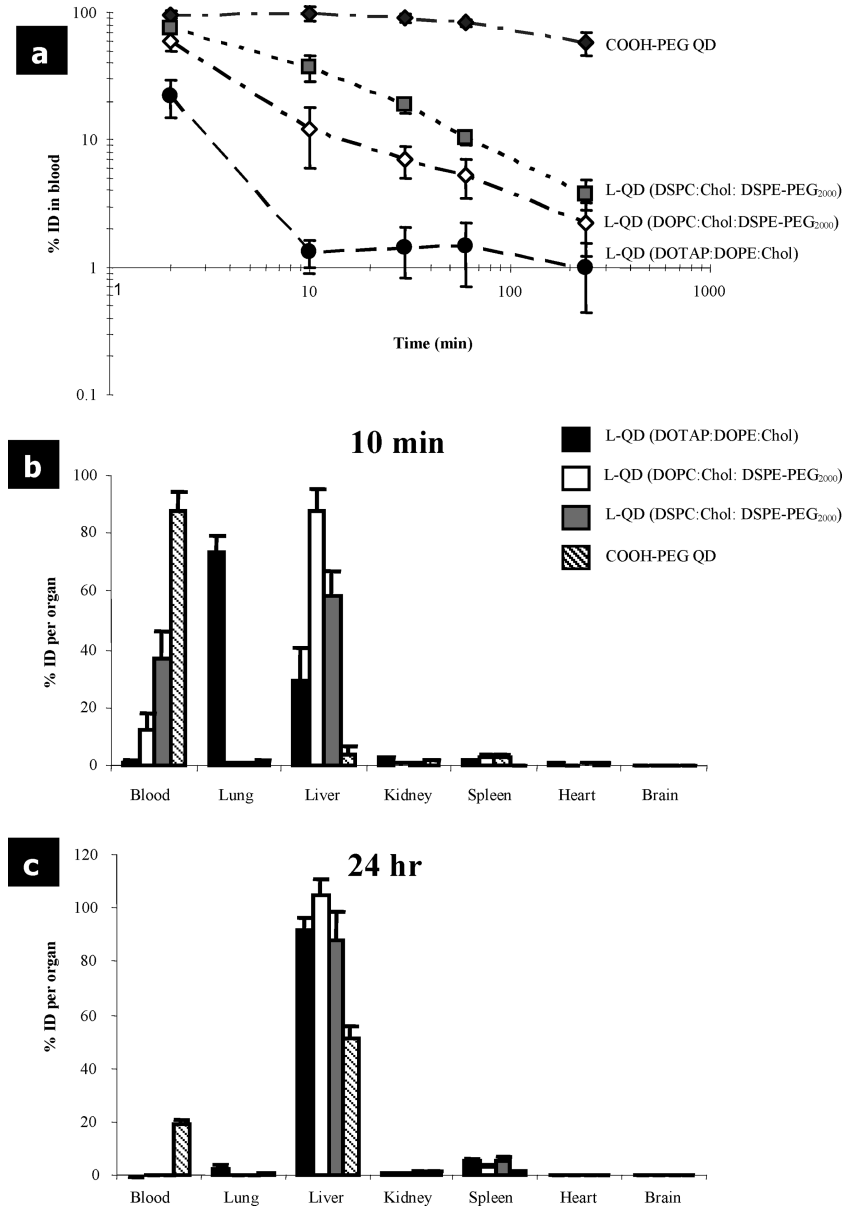

Figure 4. Biodistribution of different QD nanoparticles in CD-1 nude mice after intravenous administration. (a) Blood clearance profile of L-QD DOTAP/DOPE/Chol (2:1:1.5, circles), L-QD DOPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2, empty diamonds), L-QD DSPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2, squares), and COOH-PEG QD (filled diamonds) in CD-1 nude mice after intravenous administration. Organ biodistribution in CD-1 nude mice of L-QD DOTAP/DOPE/Chol (2:1:1.5, black bars), L-QD DOPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2, white bars), L-QD DSPC/Chol/DSPE-PEG$_{2000}$ (1.8:1:0.2, gray bars), and COOH-PEG QD (hatched bars) nanoparticles (b) 10 min and (c) 24 h after tail vein injection. Data represent the mean ± SD ($n = 4$).

recently, Frangioni et al. reported that the QD surface charge had a profound effect on the ensuing size of QD under physiological conditions. Purely negative or positive QD surface character was associated with an overall increase in the QD hydrodynamic diameter above 15 nm, due to interaction with serum proteins. This increased QD size did not allow their renal excretion and enhanced their accumulation in the liver. That study indicated that only neutral organic coatings (e.g., cysteine) that maintained the QD size in the blood below 5.5 nm were rapidly excreted in the urine and eliminated from the body (*29, 33*).

The pharmacological profiles of sterically stabilized QD using different molecular weight PEG have also been studied. QD conjugated to low molecular weight PEG (PEG$_{750}$) exhibited short blood circulation ($t_{1/2}$ < 12 min) with predominant uptake in the liver, spleen, lymph nodes, and bone marrow (*28*). Increasing PEG molecular weight to 2000 and 5000 Da significantly reduced macrophage recognition and consequently increased their blood circulation $t_{1/2}$ to an average of 5 and 18.5 h, respectively (*11, 29, 34*). Interestingly, similar observations in relation to the size of PEG on the QD surface have also been illustrated after conjugation of ligands (such as epidermal growth factor and RGD) at the distal end of the PEG-coated QD to improve their systemic tumor targeting (*35, 36*). It would be interesting to explore any differences in the pharmacokinetic profile of the L-QD systems after conjugation of targeting ligands on their lipid bilayer.

In the present study, cationic L-QD (DOTAP/DOPE/Chol) hybrid vesicles exhibited a different *in vivo* profile compared to previously reported 25 nm, cationic mercaptoundecanoic acid cross-linked with lysine-QD (*27*). Cationic L-QD hybrids cleared very rapidly from blood circulation after injection (2 min) (Figure 4b) and ~80% ID resided in the lung compared to 2%

ID of lysine-QD localized in the lung between 15 and 90 min. The low lung accumulation of lysine-QD can be explained by their small size (*37*) and lower cationic surface charge (*38*) compared to the highly positively charged L-QD hybrid vesicles (+47.5 mV). On the basis of this pharmacological profile, improvements in the dynamics of cellular uptake of cationic L-QD by the pulmonary endothelium by inclusion of an appropriate ligand may offer even more effective and specific delivery of QD to the lung tissue for imaging and tracking purposes.

CONCLUSION

In this work, we rationally modulated the surface and bilayer characteristics of the recently developed L-QD hybrid vesicles and correlated them to blood stability (*in vitro* and *in vivo*) and tissue biodistribution in mice after intravenous administration. Sterically stabilized L-QD hybrid vesicles have shown high serum stability *in vitro* comparable to corresponding liposome-alone systems. The *in vivo* profiles obtained for the different L-QD hybrid systems (by monitoring Cd atom deposition in blood and tissues) showed identical behavior between cationic L-QD and previously described cationic nanoparticles. However, in the case of PEGylated L-QD much shorter blood circulation times were observed compared to PEGylated liposomes of the same lipid composition. Such findings indicate that liposome−nanoparticle hybrid systems should not be expected to behave pharmacologically similarly to liposome-alone systems. Eventually (time points at 24 h or later), both the L-QD and functionalized QD accumulated primarily in the liver, which may cause toxicological concerns that will have to be overcome before further clinical development. Overall, the L-QD hybrid vesicle system in comparison to functionalized QD is considered a viable platform that allows the facile modulation of the hybrid vesicle characteristics for the rational design of QD delivery to different tissues such as the lung, particularly if therapeutic agents are encapsulated in the L-QD internal aqueous phase.

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Supporting Information Available: L-QD hybrid vesicle preparation, serum stability, carboxyfluorescein release, L-QD biodistribution *in vivo*, and quantification in organs. This material is available free of charge via the Internet at http://pubs.acs.org.

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