Nanotoxicology, March 2010; 4(1): 98–105

informa
healthcare

Pulmonary thrombosis in the mouse following intravenous administration of quantum dot-labeled mesenchymal cells

YUVAL RAMOT[1], MICHAL STEINER[2], VERED MORAD[2], SIGALIT LEIBOVITCH[2], NETANEL AMOUYAL[2], MARK F. CESTA[3], & ABRAHAM NYSKA[4]

[1]*Hadassah – Hebrew University Medical Center, Jerusalem, Israel,* [2]*Harlan Biotech Israel Ltd, Rehovot, Israel,* [3]*Cellular and Molecular Pathology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA, and* [4]*Consultant in Toxicological Pathology, Sackler School of Medicine, Tel Aviv University, Israel*

(Received 25 July 2009; accepted 5 November 2009)

Abstract

Quantum dots (QDs) are emerging as novel diagnostic agents. Yet, only a few studies have examined the possible deleterious effects of QD-labeled stem cells. We assessed the potential toxic effects of QD-labeled human embryonic palatal mesenchymal (QD-HEPM) cells in male NOD/SCID mice for six months, following the administration of a single intravenous injection. Control animals were administered with non-labeled HEPM cells. No treatment-related clinical signs, hematological, or biochemical parameters were found in the QD-HEPM animals in comparison to control animals. Histologically, multifocal organizing thrombi were noted in the pulmonary arteries of all QD-HEPM animals from the one-week study group and in one animal from the one-month group. Additionally, increased severity of perivascular inflammation was noted at the injection sites of QD-HEPM animals from the one-week group. This is the first study reporting histopathological evidence for pro-thrombotic adverse effects mediated by QD labeling.

Keywords: *Pulmonary thrombosis, quantum dots, NOD/SCID mouse, mesenchymal stem cells*

Introduction

Nanoparticles are currently being investigated as novel intravascular probes for diagnostics and therapeutics. They have potential applications for *in vivo* biomedical imaging and drug delivery systems (Akerman et al. 2002). The nanotechnology field is rapidly developing, and information about safety and potential hazards is urgently needed (Walker and Bucher 2009). Not surprisingly, nanotoxicology is a new emerging discipline in toxicology (Oberdörster et al. 2005). Although often regarded as a uniform group, engineered nanomaterials should not be regarded as such. These materials have different sizes, shapes, surface areas, chemical compositions and biopersistence, dictating that the possible environmental and health impact be assessed for each type of nanomaterial separately (Hoet et al. 2004).

One class of engineered nanoparticles is quantum dots (QDs), which are composed of atoms from groups II–VI or III–V in the periodic table, and defined as particles with physical dimensions smaller than the exciton Bohr radius (Chan et al. 2002; Rouse et al. 2008). This small size leads to the effect of quantum confinement, which gives rise to unique optical and electronic properties (Han et al. 2001; Chan et al. 2002). In addition, their large surface area-to-volume ratio makes them attractive for the design of more complex nanosystems (Akerman et al. 2002). Their extraordinarily intense and photostable fluorescence properties, when compared with conventional organic fluorophores, make them perfectly appropriate for use as diagnostic and imaging agents (Hsieh et al. 2006; Rouse et al. 2008).

Several *in vitro* studies suggest that certain QD types may be cytotoxic (Hardman 2006). For example, CdTe QDs were shown to be toxic to rat pheochromocytoma cells in culture (at concentrations of 1–10 μg/ml) (Lovric et al. 2005). Hoshino et al. (2004b) have shown that incubation of EL-4 cells with CdSe/ZnS–SSA QDs caused decreased cell viability at concentrations above 0.1 mg/ml. Shiohara et al. (2004) reported that mercaptoundecanoic acid coated CdSe/ZnS QDs at concentrations of 100 μg/ml were cytotoxic to HeLa cells and primary human hepatocytes. These effects were considered, at least

Correspondence: Dr A. Nyska, Haharuv 18, PO Box 184, Timrat, Israel 36576. Tel: +972 4 654 7846. Fax: +972 4 654 7846. E-mail: anyska@bezeqint.net

ISSN 1743-5390 print/ISSN 1743-5404 online © 2010 Informa UK Ltd.
DOI: 10.3109/17435390903470093

partially, to be due to the presence of Cd, formation of free radicals, and interaction with intracellular components leading to loss of function (Hoshino et al. 2004b; Shiohara et al. 2004; Lovric et al. 2005; Hardman 2006). *In vivo* studies, however, have generally reported a lack of toxicity (Larson et al. 2003; Ballou et al. 2004; Kim et al. 2004; Choi et al. 2007; Gopee et al. 2007; Yang et al. 2007). A caveat to be considered, however, is that these studies were not specifically performed to evaluate toxicity (Geys et al. 2008).

One suggested application for QDs is imaging in stem cell therapy (Lin et al. 2007). In stem cell therapy, it is necessary to efficiently monitor cell survival and biodistribution after transplantation, and finding a reliable method for stem cell tracking is of a key importance (Seleverstov et al. 2006). For QDs to be useful cell markers, it is important that they do not interfere with normal cellular physiology (Hsieh et al. 2006). Previous studies have shown that introduction of QDs into cells has no obvious cytotoxic effects, as evidenced by morphological observations and cell growth analyses (Hsieh et al. 2006). However, most QD applications have used non-mammalian or cancer cells, with only a few studies examining deleterious effects of QDs in stem cells (Muller-Borer et al. 2007). Therefore, in the present study, we assessed the potential toxic effects of QD-labeled human embryonic palatal mesenchymal (QD-HEPM) cells in various tissues at different time-points, following a single intravenous (IV) injection to male NOD/SCID mice. For the delivery of QDs into the live cells, we used the polyarginine peptide-based QTracker reagents (Invitrogen, CA, USA). These reagents combine QDs with a custom targeting peptide to improve QD solubility and intracellular delivery (Muller-Borer et al. 2007).

Materials and methods

HEPM cell culturing

HEPM cells (ATCC, Manassas, VA, USA), which are osteoblast precursor cells, were grown in 175 cm^2 culture flasks, kept at 37°C in an atmosphere of 5% CO_2 and 95% humidity. The cultured medium was composed of Eagle's minimum essential medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acid, sodium bicarbonate 1.5 g/l, heat-inactivated fetal bovine serum 10%, 100 U/ml penicillin and 100 μg/ml streptomycin (Biological Industries, Beit Haemek, Israel).

Preparing HEPM cells for injection (control group)

On the day of injection, HEPM cells were detached using trypsin-EDTA (Biological Industries, Beit Haemek, Israel), centrifuged at 1200 rpm for 10 min and resuspended in growth medium diluted 1:10 in PBS (Biological Industries, Beit Haemek, Israel). Cells were filtered in a 70 μm-pores cell strainer, counted, and re-concentrated to 10^7 cells/ml.

Labeling HEPM cells with QDs

Trypsinized HEPM cells were labeled with a Qtracker® 525 Cell Labeling Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Briefly, 30 nM of labeling solution was prepared according to kit instructions. 1.35×10^7 cells were added to the labeling solution and incubated for 1 h at 37°C, 5% CO_2 and 95% humidity. At the end of the incubation period, cells were washed twice with growth medium and filtered in a 70 μm-pores cell strainer and resuspended in growth medium diluted 1:10 in PBS to achieve a final concentration of 1×10^7 cells/ml.

Animals, treatments, and experimental procedures

Male NOD/SCID mice (strain NOD.SCID/NCrHsd-Prkdc^{scid}) of about 6–9 weeks of age were obtained from Harlan Laboratories (Rehovot, Israel) and maintained on standard chow (Harlan Teklad diet 2018S, WI, USA). They were allowed free access to drinking water, supplied to each cage via polyethylene bottles with stainless steel sipper tubes. The water was filtered (0.1 μ filter), chlorinated and acidified. During the acclimation and throughout the entire study duration, animals were housed within a limited access rodent facility and kept in groups of maximum five animals/cage in polypropylene cages (36.5 × 20.7 × 14.0 cm), fitted with solid bottoms and filled with wood shavings as bedding material (7093 Harlan Teklad Shredded Aspen). They were allowed a six-day acclimation period to facility conditions (20–24°C, 30–70% relative humidity, and a 12-h light/dark cycle) prior to inclusion in the study. Animal care and administration of HEPM cells were conducted at a GLP-certified site (Harlan Biotech Israel Ltd., Rehovot, Israel), and approved by the Committee for Ethical Conduct in the Care and Use of Laboratory Animals of the Hebrew University, Jerusalem, Israel.

The study included eight groups, four treatment groups and four control groups consisting of five male mice/group. One treated group and one control group

were subjected to sequential study termination at one week, one month, three months and six months after dosing (Table I). In all instances, QD-HEPM cells were administered at a single dose of 2×10^6 cells at a constant dose volume of 0.2 ml/animal. The control groups were injected with non-labeled HEPM cells under identical experimental conditions. The cells were administered by a single IV injection into the tail veins. The IV route of administration was selected to obtain maximal distribution of the cells.

Animals were assigned to the various study groups according to a computer-generated randomization output. Body weights were measured at randomization, prior to the first injection, and weekly thereafter. The last body weight determination was carried out prior to scheduled termination. All mice were observed for abnormal clinical signs once weekly and for morbidity and mortality once daily (six days/week). Observations included changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions (e.g., diarrhea) and autonomic activity (e.g., lacrimation, salivation, piloerection, unusual respiratory pattern). Changes in gait, posture and response to handling, as well as the presence of bizarre behavior, tremors, convulsions, sleep and coma were also observed and recorded. Animals were euthanized by CO_2 asphyxiation prior to the scheduled necropsy.

Hematology and biochemistry

Blood for hematology and biochemistry parameters was collected just prior to euthanasia and following food deprivation of at least 3 h. Blood samples of whole blood collected into EDTA-coated tubes for hematology and serum collected into non-coated tubes for biochemistry, were obtained by retro-orbital sinus bleeding under light CO_2 anesthesia. The tubes were kept at 2–8°C until transported to the analytical laboratory. The samples were assayed for hematology using the Sysmex KX-21 Hematology Analyzer (Kobe, Japan) and for biochemistry using the Roche/Hitachi Modular P800 analyzer (Roche Diagnostics, Almere, The Netherlands).

Necropsy and tissue handling

Complete necropsy and macroscopic examinations were performed on all treated and control animals. Samples from the following tissues and organs were collected and fixed in 4% formaldehyde solution: adrenals; brain; cecum; colon; duodenum; epididymides; femur and bone marrow; heart; ileum; injection site (tail); jejunum; kidney; liver; gall bladder; lungs; mesenteric lymph nodes; skeletal muscle (left thigh); spleen; stomach; and testes. Tissues were trimmed, embedded in paraffin, sectioned at approximately 5 μm thickness, and stained with Hematoxylin & Eosin (H&E). The injection site (tail) was trimmed in the middle and on both sides (proximal and distal) at about 2 mm from the middle section. All of the prepared tissue sections were examined microscopically. The brain, epididymides, heart, kidneys, liver, lungs, spleen and testes were weighed immediately following their dissection.

Lesion grading

The severity of pulmonary thrombosis was scored using a semi-quantitative grading scale, taking into consideration the relative number of vessels affected: 0 = no lesion; 1 = minimal change, up to three vessels affected; 2 = mild change, 4–10 vessels affected.

Table I. Experimental study design.

		Treatment			
Group no.	No. of animals per group	Test material	Dose (cells/0.2 ml)	Frequency and route	Study period
1	5	HEPM cells	2×10^6	Single IV injection	1 week
2	5	HEPM cells	2×10^6	Single IV injection	1 month
3	5	HEPM cells	2×10^6	Single IV injection	3 months
4	5	HEPM cells	2×10^6	Single IV injection	6 months
5	5	HEPM-QD cells	2×10^6	Single IV injection	1 week
6	5	HEPM-QD cells	2×10^6	Single IV injection	1 month
7	5	HEPM-QD cells	2×10^6	Single IV injection	3 months
8	5	HEPM-QD cells	2×10^6	Single IV injection	6 months

Statistical analysis

Data analysis of all measurable parameters was performed using a two-tail *p*-value test.

Results

Clinical observations

No mortality occurred in any of the animals prior to the scheduled study termination. No noticeable clinical signs were evident in any of the animals throughout the entire study period.

Body and organ weights

No treatment-related changes in mean body weight, mean body weight gain, or mean calculated percentage change in body weight were noted. In addition, no treatment-related changes were noted in mean organ weight or organ-weight-to-body-weight ratio.

Hematology and biochemistry

There were no treatment-related effects on any of the hematological or biochemical parameters measured in this study.

Macroscopic findings

No gross pathological findings were evident in any of the treated animals at the time of their scheduled necropsy, excluding slightly enlarged thymus which was noted in two control animals that were subjected to necropsy 1 and 6 months post-dosing (Group 2 and 4, respectively) and one QD-HEPM animal that was subjected to necropsy six months post-dosing (Group 8). Enlargement of the thymus is a common pathological finding in the NOD/SCID mouse strain and is attributed to malignant lymphoma that develops spontaneously in this strain of mouse.

Histopathological findings

Treatment-related changes were limited to the lungs and injection site of the first sacrifice period (i.e., one-week interim sacrifice), and to the lungs of the one-month interim sacrifice (Table II). No treatment-related changes were seen at the three-month and six-month sacrifice periods.

One-week sacrifice

Multifocal organizing thrombi were noted in the pulmonary arteries of all animals of the QD-HEPM group, with severity ranging from minimal to mild. The arteries were of medium to large caliber, and the lumen was partially to almost entirely occluded by a maturing thrombus (Figure 1A–C). The thrombi predominantly consisted of irregular collections of fibrin, surrounded by smooth muscle cell proliferation. Sporadically, the presence of erythrocytes, polymorphonuclear cells and macrophages was noted within the thrombus. The organized masses of thrombi were usually covered by a single layer of flattened endothelial cells. At the injection site, an apparent increased severity (from minimal to mild), when compared to the control group, of subchronic perivascular inflammation was noted.

Table II. Incidence of pulmonary histopathologic findings in the different sacrifice periods.

	Mean severity in affected animals (Number affected/Total number of animals[a])	
Lesion	HEPM cells	HEPM-QD cells
	1 week following a single injection	
Blood vessels – organizing thrombosis, multifocal	0.0 (0/5)	1.6 (5/5)
	1 month following a single injection	
Blood vessels – organizing thrombosis, multifocal	0.0 (0/5)	1 (1/5)
	3 months following a single injection	
Blood vessels – organizing thrombosis, multifocal	0.0 (0/5)	0.0 (0/5)
	6 months following a single injection	
Blood vessels – organizing thrombosis, multifocal	0.0 (0/5)	0.0 (0/5)

[a]$n = 5$ animals for each treatment group.

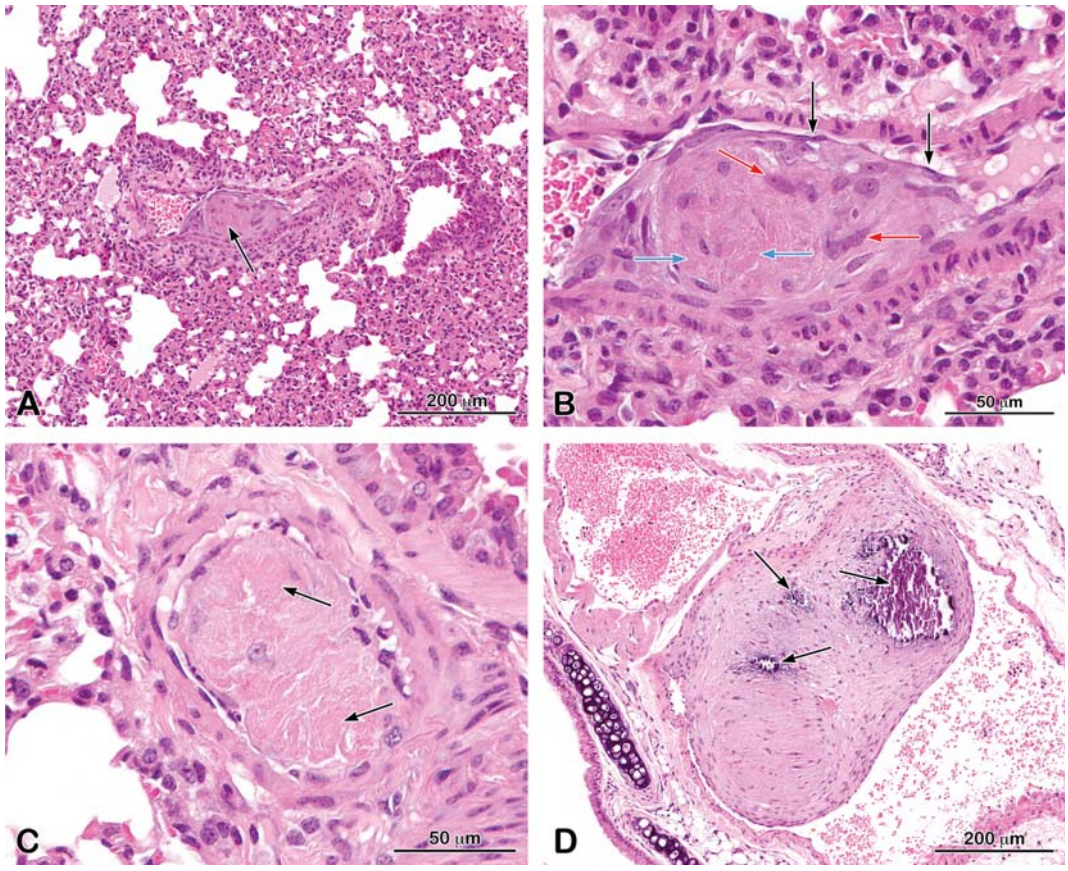

Figure 1. (A) Medium caliber pulmonary artery from the IV QD-HEPM group sacrificed one week following injection. The lumen is partially occluded by a maturing thrombus (arrow). The thrombus consists of irregular collections of fibrin, surrounded by a smooth muscle cell proliferation. H&E. (B) Same sample as presented in (A) at higher magnification. The thrombus consists of irregular collections of fibrin (blue arrows), surrounded by smooth muscle cell proliferation (red arrows). The organized masses of thrombi are usually covered by a single layer of flattened endothelial cells (black arrows). H&E. (C) Medium caliber pulmonary artery from the IV QD-HEPM group sacrificed one week following injection. The lumen is almost completely occluded by a maturing thrombus. The thrombus consists of irregular collections of fibrin (arrows), surrounded by smooth muscle cell proliferation. H&E. (D) Large caliber pulmonary artery from the IV QD-HEPM group sacrificed one month following injection. The lumen is partially occluded by a maturing thrombus, which consists of mature, poorly nucleated mesenchymal tissue and irregular collections of mineralized granules (arrows). H&E.

One-month sacrifice

In a single mouse from the treated group (i.e., QD-HEPM), an organizing thrombus was noted in a pulmonary artery (Figure 1D). Compared to the multiple thrombi noted in affected animals at the one-week sacrifice, the thrombus was noted only in one large caliber vessel. It consisted of mature scarring, relatively poorly nucleated tissue, and irregular collections of mineralized granules. At the injection site, only few cases of minimal sub-chronic inflammation were noted in the treated group.

The lack of treatment-related changes in the three-month and six-month sacrifices is indicative of a complete recovery at these time periods.

Discussion and conclusion

Stem cells present an exciting new therapeutic modality to treat a variety of conditions and diseases. Therefore, it is important to find methods for monitoring their survival and biodistribution after transplantation. Because of their many advantages over conventional organic dyes, QDs are good candidates to monitor these parameters (Lin et al. 2007). In a pioneering study by Jaiswal et al. (2003), the authors showed that QDs can be internalized by live cells (HeLa cells), allowing tracking of these cells for more than 10 days, and likewise demonstrated that these cells grew and developed normally without any morphological signs of toxicity. Accordingly, it was anticipated that this method can be used for long-term

imaging studies and drug deliveries without any evident toxicity (Ryman-Rasmussen et al. 2007). Indeed, following this study, several other experiments have demonstrated that the use of QDs is safe, and that labeling of cells with these nanocrystals neither interferes with normal cell physiology nor causes any adverse effects on cells (Jaiswal et al. 2003; Chen and Gerion 2004; Voura et al. 2004; Hsieh et al. 2006; Lin et al. 2007). In view of these findings it was suggested that at the concentrations used for *in vivo* applications, QDs are safe and thus constitute a well-tolerated means of labeling stem cells (Lin et al. 2007).

However, in the few last years, there have been increasing reports of QD cytotoxicity in the literature. Lovric et al. (2005) have shown that QDs can cause cell death in rat pheochromocytoma cells, characterized by chromatin condensation and membrane blebbing. It was suggested that this toxicity may be mediated by free Cd, liberated as a result of QD deterioration, which leads to the formation of free radicals (Lovric et al. 2005). Indeed, in a different study, cytotoxicity was found to correlate with the liberation of free Cd^{2+} ions (Derfus et al. 2004). Another proposed mechanism for toxicity is interaction of nanoparticles with individual cellular components, leading to their malfunction and ultimately, to cell death (Lovric et al. 2005).

Using cell viability assays, which measured the amount of hormazan generated following exposure to tetrazolium salt, mercapto-undecanoic acid-coated CdSe/ZnS QDs were found to decrease cell viability with increasing concentrations (Shiohara et al. 2004). Importantly, cell viability was decreased even at low concentrations, but without leading to cell death. QDs may have also genotoxicity potential, as treatment of WTK1 cells for only 2 h led to DNA damage (Hoshino et al. 2004a). This damage was repaired after 12 h.

In our current study, we have observed increased severity of perivascular inflammation at the injection sites of QD-HEPM injected animals from the one-week group. Recently, a potential inflammatory effect has been attributed to QDs, when it was shown that carboxylic acid-coated QDs significantly increased release of IL-1β, IL-6, and IL-8 from human epidermal keratinocytes (Ryman-Rasmussen et al. 2007). In line with these results, MCP-1 and IL-6 were also found to be elevated after QD labeling of mesenchymal stem cells (Muller-Borer et al. 2007). Additionally, the administration of QDs to mesenchymal stem cells may hinder their proper differentiation process. For example, internalized QDs were found to interfere with the induced chondrogenesis of mesenchymal stem cells, as indicated by the suppression of mRNA and protein expression of chondrocyte-specific markers (Hsieh et al. 2006). The administration of QDs may lead to cellular stress, causing the activation of autophagy, and to damaged mitochondria (either a primary event, which leads to the initiation of autophagy, or as a consequence of autophagy) (Seleverstov et al. 2006). This mechanism of cellular defense was more evident in small-sized QDs (similar to the ones used in our current study). However, no significant metabolic or proliferative function impairments were noted, suggesting that the cells can replace damaged organelles (Seleverstov et al. 2006).

Although some of these *in vitro* studies suggested potential cytotoxicity, they do not necessarily predict the behavior of cells under *in vivo* conditions. For example, application of QDs to lymphoma cell lines led to decreased cell viability, but when these cells were injected into mice, no apparent adverse effects were noted (Hoshino et al. 2004b). Therefore, additional knowledge on the potential toxic effects of QDs in *in vivo* studies is of paramount importance. This is especially true considering that the properties of these recently created nanomaterials could lead to new biological interactions and result in unanticipated toxicity (Walker and Bucher 2009).

In the current study, we observed pulmonary thrombosis after IV administration of QD-HEPM cells. The thrombi were restricted to the pulmonary arteries, and were not seen in other vessels. It is anticipated that, when administered IV, the majority of stem cells are initially trapped in the lungs (Fischer et al. 2009). The stem cells, when circulating to the lungs, may not be able to pass through the narrow pulmonary capillaries or may adhere to endothelial cells in a receptor-mediated route (Gao et al. 2001). This trapping of stem cells in the 'lung barrier' may potentially lead to hemodynamic alterations (Gao et al. 2001). In the present study, non-QD labeled HEPM cells did not lead to thrombi in the lung vessels, whereas thrombi were clearly evident in all of the animals treated with QD-labeled HEPM cells at one-week post-injection. This suggests that the delivery of QD to HEPM cells caused changes in intracellular physiology thereby rendering them more prone to pulmonary stasis and subsequent induction of local thrombosis formation.

Some of these effects may be correlated with the known cytotoxic effects of QDs. For example, Cd release inside cells may induce the formation of reactive oxygen species (Lovric et al. 2005) and lead to increased expression of VLA-4 (Tatara et al. 2009), which in turn may mediate increased adherence of stem cells to pulmonary arterial endothelium (Fischer et al. 2009). The secretion of inflammatory cytokines from QD-containing cells (Muller-Borer et al.

2007; Ryman-Rasmussen et al. 2007), may result in endothelial cell activation due to cytokine-mediated activation of the coagulation cascade (Esmon 2005; Ramot and Nyska 2007). Additionally, QDs may lead to decreased cell motility, thereby contributing to cell stasis in the capillaries (Hardman 2006). All of these potential mechanisms may ultimately lead to an increased tendency towards thrombosis. It can not be excluded that some of the adverse effects observed in the current study could have stemmed from the influence of the labeling procedure itself on the cells, and not from the mere presence of QDs in the labeled cells.

The thrombi observed in our current study were of limited (minimal to mild) severity. It is important to note that these thrombi underwent spontaneous regression, as after one month only one animal still showed evidence of thrombi in the pulmonary vessels, and after three months, none of the animals demonstrated any evidence of thrombosis in the lungs or any other organ. Furthermore, no clinical abnormality was evident in any of the treated animals, and no differences in blood parameters were noted between control and treated animals (e.g., no differences in platelet counts).

It should be made clear that QDs are not all alike (Hardman 2006). Each QD has its own unique physicochemical characteristics, which determine its potential toxicity or lack thereof (Hardman 2006). In the current study we utilized small particles (QD525), which are known to be more toxic than larger ones, possibly because of the smaller aggregates formed (Lovric et al. 2005). Therefore, conclusions from the current study should not be generalized to any other QD types.

The morphological features of the organized thrombi noted in the present study, resemble those of the so-called "arterial plaques" that are known to occur spontaneously in the lungs of various strains of mice (Rehm et al. 1985; Ernst et al. 1996). Interestingly, the investigators have suggested that these plaques probably represent thrombi in different stages of organization (Rehm et al. 1985; Ernst et al. 1996).

In conclusion, the administration of a single IV injection of QD-HEPM cells to male NOD/SCID mice was found to be associated with minimal to mild, multifocal organizing thrombi in the pulmonary arteries of all treated animals at one week post-injection and in one animal at one-month post-injection. No thrombi were noted in animals of the three-month study group. This study, undertaken in order to assess the *in vivo* toxicity of QD-labeling of stem cells, is the first to report histopathological evidence for pro-thrombotic adverse effects caused by QD-labeling, and to highlight the risk of unanticipated toxicity with the use of nanomaterials. In light of the potential future use of intravascularly administered QD-labeled stem cells, we suggest that additional *in vivo* testing be undertaken in order to meticulously evaluate any hemodynamic adverse effects.

Acknowledgments

The authors wish to thank Dr Yona Grunfeld from Harlan Biotech Ltd, Israel for the excellent comments. This research was supported [in part] by the Intramural Research Program of the NIH, National Institutes of Environmental Health Sciences and by Genesis Cell Therapy Consortium (A.R.), operated by the Chief Scientist Office of the Israeli Ministry of Industry, Trade and Labor (MIT).

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper and have no competing financial interests.

References

Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. 2002. Nanocrystal targeting in vivo. Proc Natl Acad Sci USA 99(20):12617–12621.

Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. 2004. Noninvasive imaging of quantum dots in mice. Bioconjug Chem 15(1):79–86.

Chan WC, Maxwell DJ, Gao X, Bailey RE, Han M, Nie S. 2002. Luminescent quantum dots for multiplexed biological detection and imaging. Curr Opin Biotechnol 13(1):40–46.

Chen F, Gerion D. 2004. Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. Nano Lett 4(10):1827–1832.

Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. 2007. Renal clearance of quantum dots. Nat Biotechnol 25(10):1165–1170.

Derfus A, Chan W, Bhatia S. 2004. Probing the cytotoxicity of semiconductor nanocrystals. Nano Lett 4(1):11–18.

Ernst H, Dungrowth D, Kamilo K, Rittinghausen S, Mohr U. 1996. Nonneoplastic lesions in the lungs. In: Mohr U, Dungrowth D, Capen C, Carlton W, Sundberg J, Ward J, editors. Pathobiology of the aging mouse. Vol. 1. Washington, DC: ILSI Press. pp 281–300.

Esmon CT. 2005. The interactions between inflammation and coagulation. Br J Haematol 131(4):417–430.

Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS Jr. 2009. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. Stem Cells Dev 18(5): 683–692.

Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. 2001. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs 169(1):12–20.

Geys J, Nemmar A, Verbeken E, Smolders E, Ratoi M, Hoylaerts MF, Nemery B, Hoet PH. 2008. Acute toxicity and

prothrombotic effects of quantum dots: Impact of surface charge. Environ Health Perspect 116(12):1607–1613.

Gopee NV, Roberts DW, Webb P, Cozart CR, Siitonen PH, Warbritton AR, Yu WW, Colvin VL, Walker NJ, Howard PC. 2007. Migration of intradermally injected quantum dots to sentinel organs in mice. Toxicol Sci 98(1):249–257.

Han M, Gao X, Su JZ, Nie S. 2001. Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. Nat Biotechnol 19(7):631–635.

Hardman R. 2006. A toxicologic review of quantum dots: Toxicity depends on physicochemical and environmental factors. Environ Health Perspect 114(2):165–172.

Hoet PH, Nemmar A, Nemery B. 2004. Health impact of nanomaterials? Nat Biotechnol 22(1):19.

Hoshino A, Fujioka K, Oku T, Suga M, Sasaki Y, Ohta T, Yasuhara M, Suzuki K, Yamamato K. 2004a. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Lett 4(11): 2163–2169.

Hoshino A, Hanaki K, Suzuki K, Yamamoto K. 2004b. Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body. Biochem Biophys Res Commun 314(1):46–53.

Hsieh SC, Wang FF, Hung SC, Chen YJ, Wang YJ. 2006. The internalized CdSe/ZnS quantum dots impair the chondrogenesis of bone marrow mesenchymal stem cells. J Biomed Mater Res B Appl Biomater 79(1):95–101.

Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. 2003. Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat Biotechnol 21(1):47–51.

Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. 2004. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 22(1):93–97.

Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW. 2003. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science 300(5624):1434–1436.

Lin S, Xie X, Patel MR, Yang YH, Li Z, Cao F, Gheysens O, Zhang Y, Gambhir SS, Rao JH, Wu JC. 2007. Quantum dot imaging for embryonic stem cells. BMC Biotechnol 7:67.

Lovric J, Bazzi HS, Cuie Y, Fortin GR, Winnik FM, Maysinger D. 2005. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. J Mol Med 83(5):377–385.

Muller-Borer BJ, Collins MC, Gunst PR, Cascio WE, Kypson AP. 2007. Quantum dot labeling of mesenchymal stem cells. J Nanobiotechnol 5:9.

Oberdörster G, Oberdörster E, Oberdörster J. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839.

Ramot Y, Nyska A. 2007. Drug-induced thrombosis – experimental, clinical, and mechanistic considerations. Toxicol Pathol 35(2): 208–225.

Rehm S, Wcislo A, Deerberg F. 1985. Non-neoplastic lesions of female virgin Han:NMRI mice, incidence and influence of food restriction throughout life span. II: Respiratory tract. Lab Anim 19(3):224–235.

Rouse JG, Haslauer CM, Loboa EG, Monteiro-Riviere NA. 2008. Cyclic tensile strain increases interactions between human epidermal keratinocytes and quantum dot nanoparticles. Toxicol In Vitro 22(2):491–497.

Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA. 2007. Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. J Invest Dermatol 127(1):143–153.

Seleverstov O, Zabirnyk O, Zscharnack M, Bulavina L, Nowicki M, Heinrich JM, Yezhelyev M, Emmrich F, O'Regan R, Bader A. 2006. Quantum dots for human mesenchymal stem cells labeling. A size-dependent autophagy activation. Nano Lett 6(12): 2826–2832.

Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K. 2004. On the cyto-toxicity caused by quantum dots. Microbiol Immunol 48(9):669–675.

Tatara Y, Ohishi M, Yamamoto K, Shiota A, Hayashi N, Iwamoto Y, Takeda M, Takagi T, Katsuya T, Ogihara T, Rakugi H. 2009. Macrophage inflammatory protein-1beta induced cell adhesion with increased intracellular reactive oxygen species. J Mol Cell Cardiol 47(1):104–111.

Voura EB, Jaiswal JK, Mattoussi H, Simon SM. 2004. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nat Med 10(9):993–998.

Walker NJ, Bucher JR. 2009. A 21st century paradigm for evaluating the health hazards of nanoscale materials? Toxicol Sci 110(2):251–254.

Yang RS, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC, Yeh TK, Yang CS, Lin P. 2007. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. Environ Health Perspect 115(9): 1339–1343.