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Bright Green Emitting Maltodextrin Coated Cadmium Sulfide Quantum Dots as Contrast Agents for Bioimaging: A Biocompatibility Study

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Abstract

The biological characteristics and physicochemical properties of quantum dots (QDs) pose new challenges in understanding, predicting, and managing their potential adverse health effects following exposure. The safe use of QDs in biomedicine requires a detailed understanding of their biocompatibility and toxicity. This study evaluates the biocompatibility of maltodextrin coated cadmium sulfide quantum dots in rodents. The *in vivo* localization of maltodextrin coated cadmium sulfide QDs in tissues was studied under fluorescence microscopy, and their biocompatibility was assessed through a histopathological analysis and biochemical assays. Animals treated daily with 200 µg/Kg of maltodextrin coated cadmium sulfide QDs for either 5 days or 15 days showed a differential presence of quantum dots in their organs. Intense fluorescence was detected in kidney, liver; muscle and brain, with moderate fluoresce detected in intestine, lung, and testis. The histopathological and biochemical analyses showed that QDs were non-toxic for rodents. The *in vivo* analysis revealed the distribution pattern of QDs and their biocompatibility with all tissues analyzed. The bright green emitting of maltodextrin coated cadmium sulfide QDs suggests they might be used as contrast agents for bio-imaging applications.

Keywords: Quantum dot; Cadmium sulfide; Cytotoxicity; Apoptosis; Biocompatibility

Introduction

Quantum dot nanoparticles (QDs) are emerging as a new contrast agent that can be employed in the biological system due to their potential use in imaging and therapeutic applications [1,2]. The unique optical properties of QDs for *in vivo* imaging include: a high absorption coefficient, a high fluorescence quantum yield, and high resistance to photo bleaching. More importantly, the broad absorption and narrow emission spectra of QDs make them suitable for simultaneous multiplex imaging [3]. However, the incorporation of QDs into biological systems often requires strategies for the manipulation of the ligands bound to the surface of the QDs in order to make them water-soluble [4].

The usefulness of QDs in biomedical applications requires that they enter the body and contact tissues and cells directly. This perforce requires knowledge of their biocompatibility. QDs are considered biocompatible that is, compatible with living tissues or a living system when they are neither toxic nor injurious or physiologically reactive [5]. By the same token, QDs are considered toxic when the particles adversely affect the normal physiology and/or directly alter the normal structure of human and animal organs and tissues [6,7].

Scientific knowledge regarding the cell-interaction mechanisms of QDs has grown in recent years. Previous results strongly suggest that surface coatings can improve cytocompatibility and consequently, decrease toxicity. Some studies have shown that long-term exposure of surface coated QDs to their bio environment can destabilize the binding strength of the surface molecules, which in turn can result in unprotected QDs inside or outside the cells [8,9]. Therefore, the stability and binding strength of surface molecules over the QD surface define the biocompatibility of the QDs and hence, their toxicity. Currently, polymers with excellent biocompatibility and low toxicity are being successfully and widely employed to modify the surface of QDs and engineer biocompatible QDs composites for a variety of medical and biological applications [10,11].

We recently reported the synthesis of size 3 nm QDs with an increased green light emitting capacity and coated them with sugar polymers, concluding they can be potentially used in the field of bioimaging [12,13]. Maltodextrin capped cadmium sulfide (CdS-MDx) QDs have produced distinct dose-dependent effects *in vitro* and *in vivo*; *in vivo* effects, however, are unknown. In this study, maltodextrin capped cadmium sulfide (CdS-MDx) QDs were subjected to a biocompatibility analysis. In order to determine the biocompatibility of CdS-MDx QDs, we histopatologically examined fluorescence emission in tissue from rodents receiving a daily dose of nanoparticles during either 5 or 15 days. Therefore, the aim of the present study was to evaluate the biocompatibility of CdS-MDx QDs *in vivo*.

Material and Methods

Analysis of *in vivo* biodistribution and biocompatibility of CdS-MDx QDs

Bright green emitting CdS-MDx QDs that were 3 nm in size were synthesized as previously described [13]. The Wistar rat was selected as the model for the biocompatibility study of CdS-MDx QDs. All animals were kept in an animal house for 12 hours day/night cycle for 2 months. All animals were kept in hygienic and animal-friendly conditions, housed in a temperature and humidity controlled environment, and allowed

food (Standard Purina Chow Diet, Mexico) and water ad libitum. The experiments were conducted in accordance with the Guide for the Care and Use for Laboratory Animals [14].

Twenty four healthy Wistar rats (8-10 weeks old) were selected randomly and divided into three groups: in one group, 8 animals were treated with a daily i.p. dose of CdS-MDx QDs in 300 µL PBS at 200 µg/Kg body weight for 5 days; in the second group, 8 animals were treated with a daily dose of CdS-MDx QDs at 200 µg/Kg body weight i.p., during 15 days, in 300 µL PBS. The control group (8 animals) was injected with 300 µL PBS. Animals were observed for signs of toxicity. Signs recorded during acute toxicity included: motor activity, anesthesia, tremor, arching, and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, exophthalmos, pilo-erection, salivation, depression, ataxia, sedation, hypnosis, cyanosis and analgesia. Behaviour parameters, death, the weight, and the amount of water and feed were analyzed. Toxicity indices consisted of daily clinical observations, body weight, food consumption, clinical pathology, organ weights, and histopathology.

After treatment, animals were fasted overnight and blood samples were obtained from the heart following anesthesia with ether. Tissues collected at necropsy were preserved in 10% neutral-buffered formalin fixative and were processed for routine histological examination. For fluorescence analysis, tissue samples were stained with H&E stain. Serum was separated by centrifugation at 3000 rpm for 15 min. Biochemical parameters of serum enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, cholesterol, triglyceride (TG), urea, creatinine and uric acid from animals that received multiple doses of CdS-MDx QDs were measured using a commercial reagent kit (ELITech, Mexico).

Statistical analysis

The data were represented as the mean ± SD of 3 independent experiments. The data was statistically analyzed using the SPSS 10.0 software (SPSS Inc., Chicago, Ill., USA), the t-test, and ANOVA. Differences were considered significant if the P- value was less than 0.05.

Results

In vivo biocompatibility of CdS-Dx QDs fluorescence microscopy

In order to obtain precise data regarding the biocompatibility of CdS-MDx QDs in rodent tissues, we treated rats with CdS-MDx QDs at 200 µg/Kg during either 5 or 15 days. The unstained tissue samples were observed under a fluorescence microscope and CdS-MDx QDs were identified because of their bright green light. The unstained tissue samples were analyzed to detect tissue distribution and accumulation. A histopathological analysis of stained tissue was also undertaken in order to monitor their toxicity and see if CdS-MDx QDs were biocompatible or if they induced structural changes. Figures 1 and 2 shows the representative fluorescence images of the biodistribution of CdS-MDx QDs in intestine, lung, spleen, heart, kidney, liver, brain, and skeletal muscle from female rats. Figures 3 and 4 show representative fluorescence images of the bio distribution of CdS-MDx QDs in intestine, lung, spleen, heart, kidney liver, brain and testis from male rats. The visualization of differing fluorescence intensity due to the presence of CdS-MDx QDs in each tissue was evident in both sexes. In intestine, the CdS-MDx QDs was more distributed in the adventitia, lamina propria and lamina epithelialis. In females, fluorescence in these tissues was more evident at 5 days. In the case of males, however, fluorescence intensity was higher at 15 days. In the lung, evident fluorescence surrounded the blood vessels, bronchioles (smooth muscle, submucose and cartilage) and alveolar sacs (luminal alveolar epithelium, smooth muscle and basement membrane); no significant changes in the intensity of fluorescence were observed in either sex. In the spleen, fluorescence intensity was high when rats were treated with CdS-MDx QDs for 5 days. The QDs were observed mainly in red pulp and, to a lesser extent, in white pulp. After 15 days of exposure, the CdS-MDx QDs were observed in the manti zone of the follicle and in the marginal zone of the follicle in female rats, whereas in males the CdS-MDx QDs were mainly found in red pulp; no significant changes in the intensity of fluorescence were observed in either sex. In the heart, CdS-MDx QDs were found in the myocardium (muscle fibers) and were less evident at 5 days of treatment in female rats.

The kidney showed more fluorescence intensity than any other organ. This was detected mainly in the proximal and distal convoluted tubule and, to a lesser degree, in the glomeruli; the bright green emitting was more intense in female rats. The liver also showed high fluorescence intensity, mostly around the hepatic portal venule, the branch of the hepatic artery, the bile duct and, to a lesser extent, the hepatic parenchyma. The bright green emitting was more intense in male rats.

CdS-MDx QDs evidently crossed the blood-brain barrier and blood-testis barrier because we detected fluorescence in brain and testis. Fluorescence was present in all layers of the cerebral cortex, but it was higher in the molecular layer in females, and in the external granular layer and the ganglionar layer in males. The testis showed intense fluorescence in Leydig cells and reduced intensity in the seminiferous tubule, as well as in the spermatocytes' zone. A high uptake of CdS- MDx QDs was observed in skeletal muscle, and the lowest degree of fluorescence was detected in the thymus (data not shown). It is interesting to note that, in most of the tissues, bright green emitting was higher after 15 days of treatment. No significant changes were observed in either female or male rats.

The histopathological analysis did not reveal any alterations in all studied tissues after 5 and 15 days of exposure to CdS-MDx QDs (Figures 5 and 6). Analyzed biochemistry parameters did not show alterations when compared with male rats in the control group (Table 1); however, an important increase (100%) in serum triglycerides levels was observed in female rats ($p<0.05$) (Table 2).

Discussion

Quantum dots have received much attention recently given their promising role in bioimaging [15,16]. However, the use of QDs for biological and clinical applications still has serious limitations because there is not enough data regarding their biocompatibility in vivo, and this has led to concerns regarding their safety [17,18]. The present study shows evidence of the biocompatibility of CdS-MDx QDs in rodents, as well as the high quality of the images that can be obtained using the CdS-MDx QDs.

The biocompatibility of QDs is one of the most important requirements for their application in bioimaging [19]. In order to make QDs more biocompatible with their biological environment, these need to be made hydrophylic. It is therefore crucial that they be rendered water-soluble through the modification of their surface. The stability of QDs in water can be obtained by either a complete ligand exchange procedure, or through steric stabilization where the native hydrophobic surface is coated with amphiphilic molecules, like polymers [20,21]. The present study used maltodextrin coated cadmium sulphide quantum dots which, as previously reported, have a size of 3 nm [13]. Since maltodextrin has been found to be safe and non-toxic, we have used it as capping agent in the synthesis of CdS QDs. Maltodextrin is considered a good coating material because it exhibits low viscosity and also shows good water solubility. Pharmaceutically, it is used as a diluent in tablets and as a coating material in microencapsulation [22]. The development of a new type

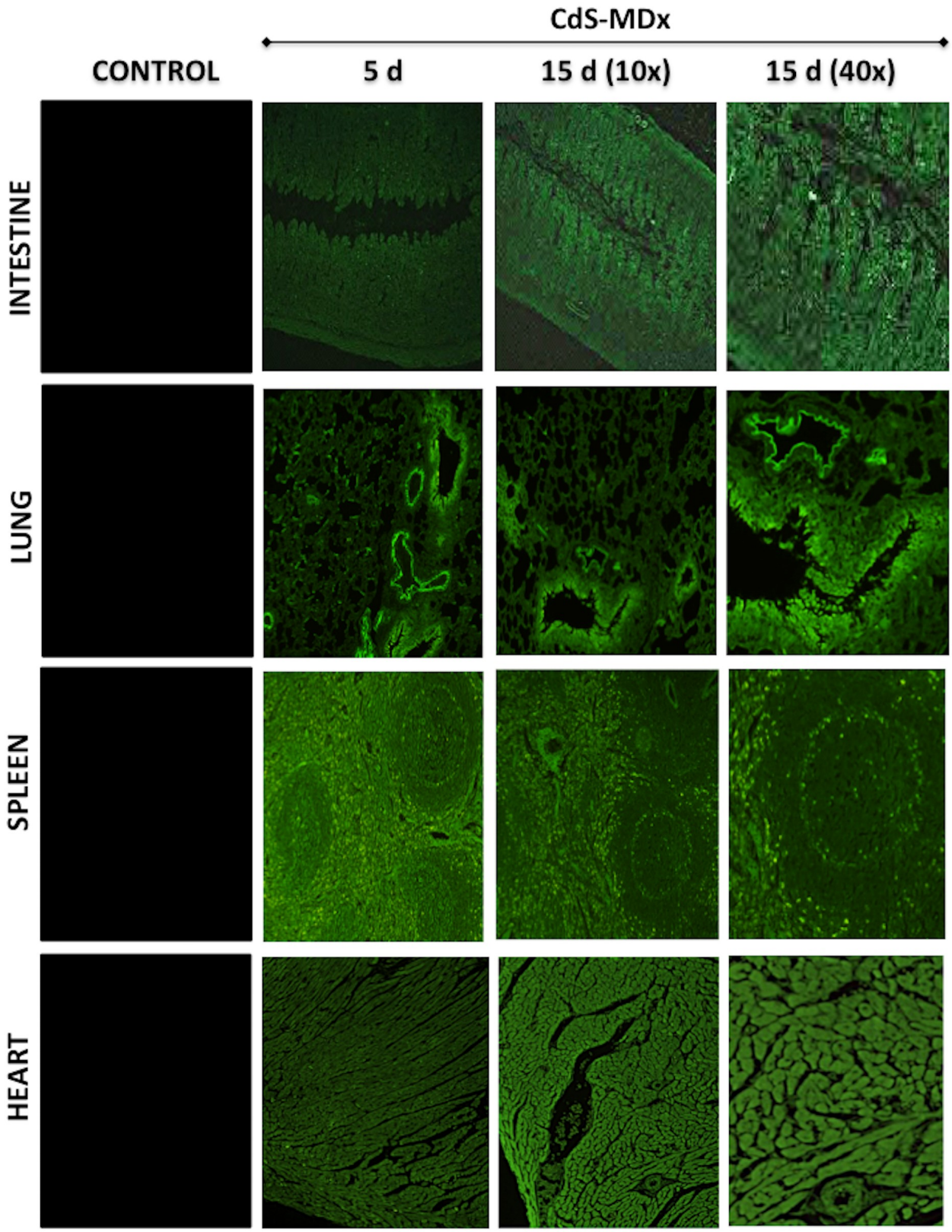

Figure 1: Fluorescence microscopy images showing the distribution and localization of CdS-MDx QDs in tissues from rats. The images belong to the intestine, lung, spleen and heart of female rats treated with 200 µg/Kg daily i.p., for 5 and 15 days. The left column shows tissues from the control group observed under fluorescence microscopy. The distribution and localization of CdS-MDx QDs was identified by a bright green imaging in the analyzed tissues. There is an evident increase in fluorescence in the intestine and heart at 15 days.

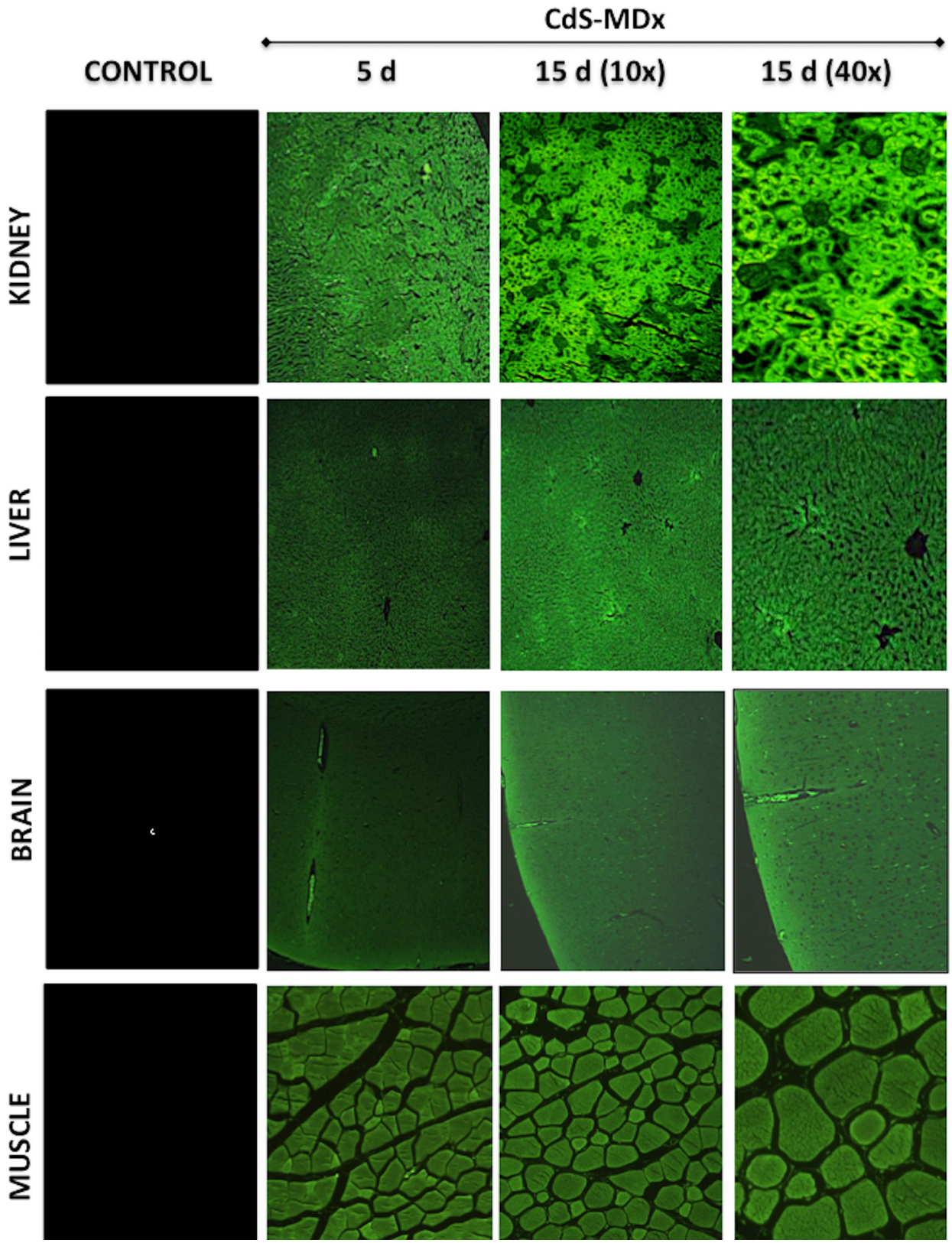

Figure 2: Fluorescence microscopy images showing the distribution and localization of CdS-MDx QDs in tissues from rats. The images belong to the kidney, liver, brain and muscle of female rats treated with 200 µg/Kg daily i.p., for 5 and 15 days. The left column shows tissues from the control group observed under fluorescence microscopy. The distribution and localization of CdS-MDx QDs was identified by a bright green imaging in the analyses tissues. There is an evident increase in fluorescence in all organs after 15 days of treatment.

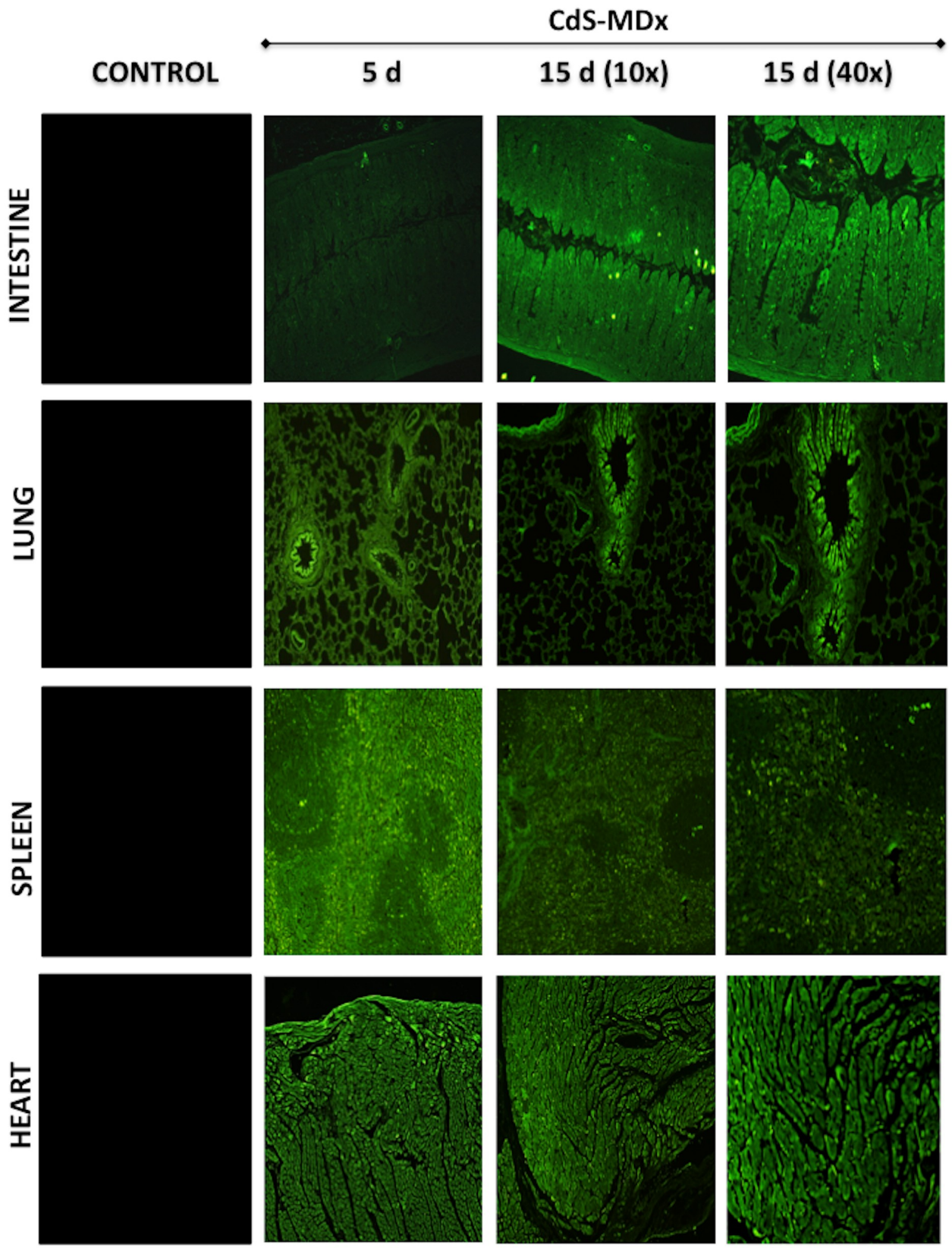

Figure 3: Fluorescence microscopy images showing the distribution and localization of CdS-MDx QDs in tissues from rats. The images belong to the intestine, lung, spleen and heart of male rats treated with 200 µg/Kg daily i.p., for 5 and 15 days. The left column shows tissues from the control group observed under fluorescence microscopy. The distribution and localization of CdS-MDx QDs was identified by a bright green imaging in the analyzed tissues.

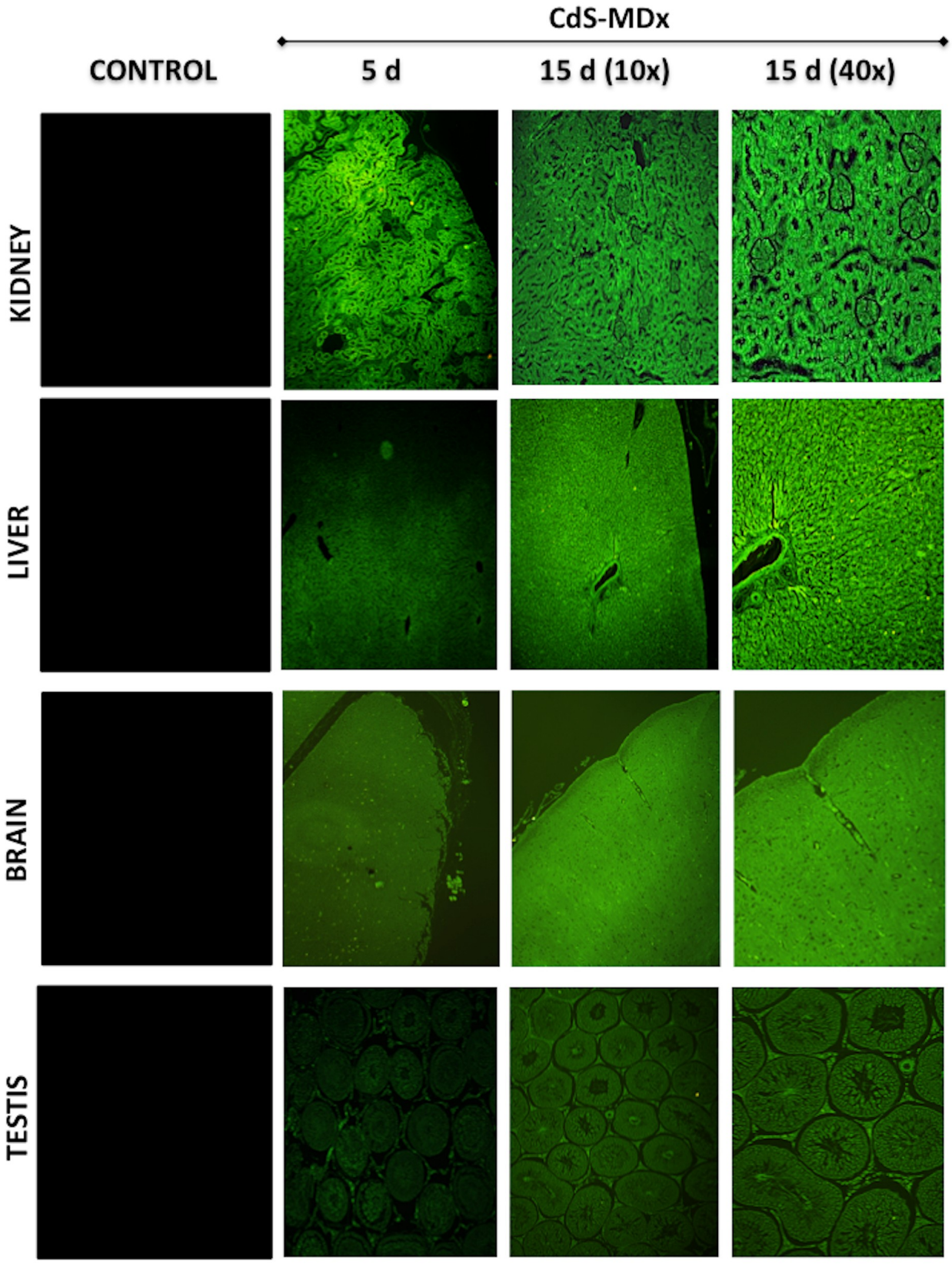

Figure 4: Fluorescence microscopy images showing the distribution and localization of CdS-MDx QDs in tissues from rats. The images belong to kidney, liver, brain and testis of male rats treated with 200 µg/Kg daily i.p., for 5 and 15 days. The left column shows tissues from the control group observed under fluorescence microscopy. The distribution and localization of CdS-MDx QDs was identified by a bright green imaging in the analyzed tissues. There was an evident increase in fluorescence in the kidney, liver and brain after 15 days of treatment.

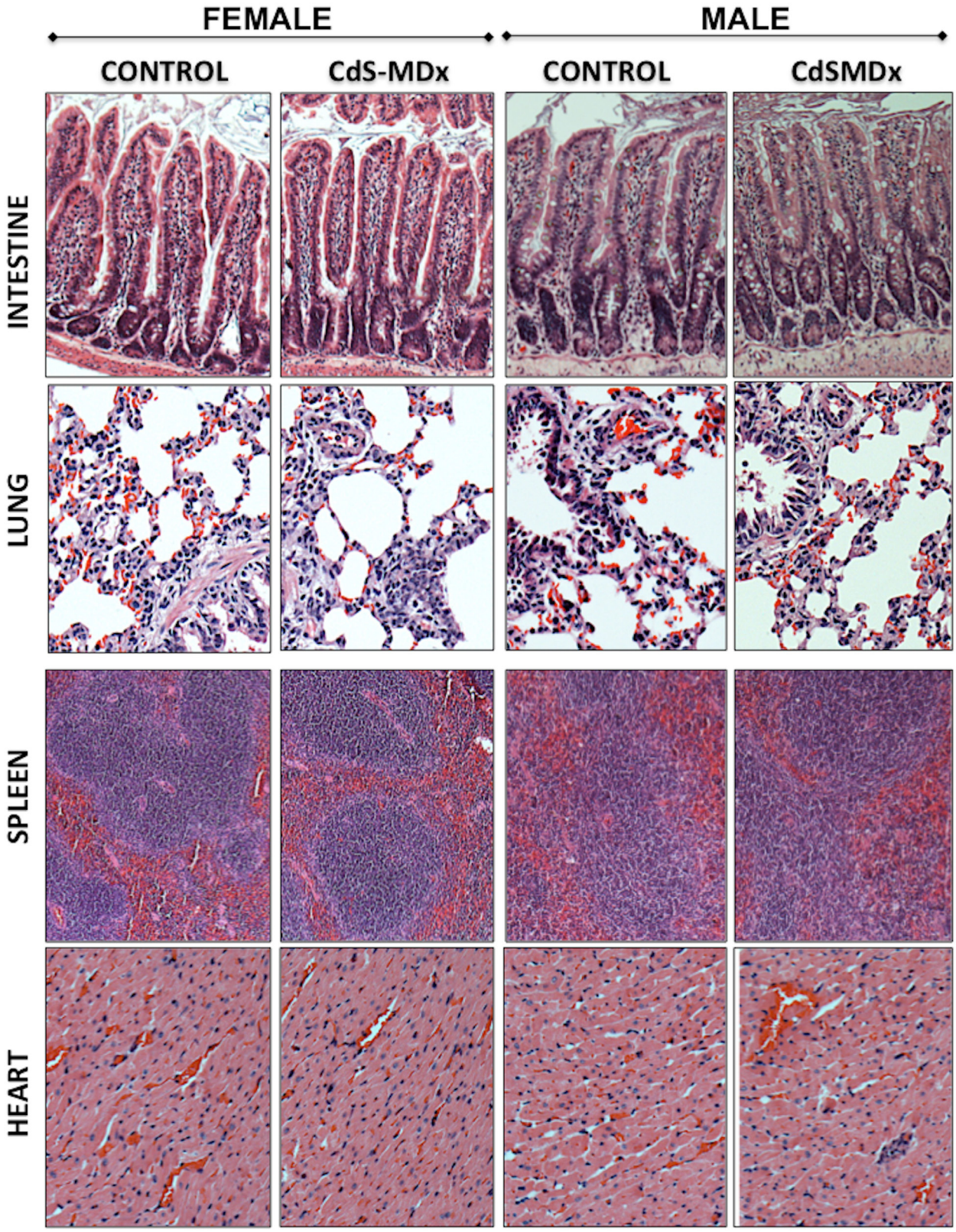

Figure 5: Representative light microscopy image of tissues from rats treated with CdS-MDx QDs. The images belong to the intestine, lung, spleen and heart from male and female rats treated with 200 µg/Kg daily, i.p., for 15 days. Our analysis shows that the organs do not exhibit signs of toxicity. The integrity of tissues was analyzed after staining with Hematoxilin & Eosin (Magnification 10X).

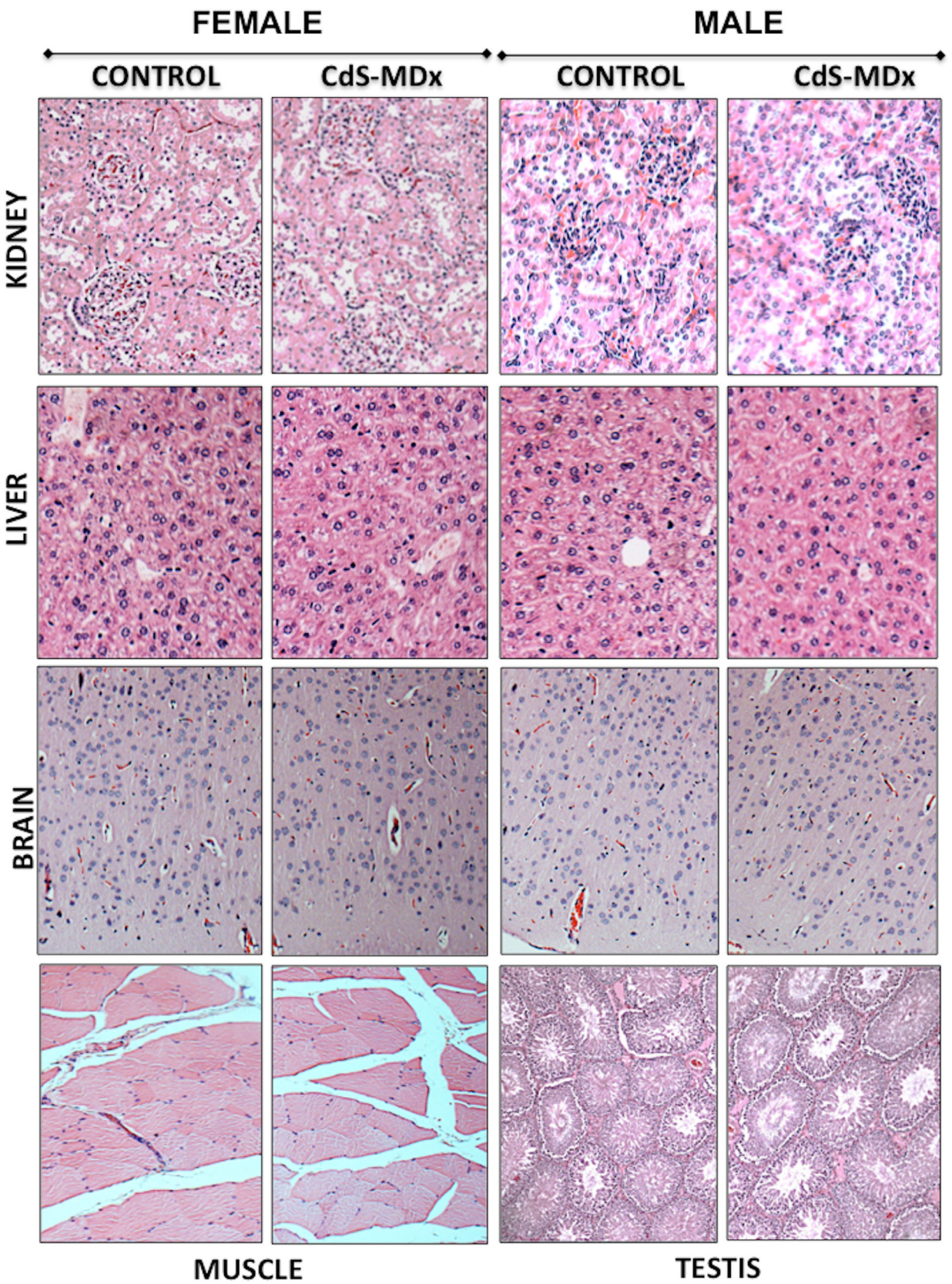

Figure 6: Representative light microscopy image of tissues from rats treated with CdS-MDx QDs. The images belong to the kidney, liver, brain, muscle and testis of rats treated with 200 μg/Kg, i.p., and daily for 15 days. Our analysis shows that the organs do not exhibit signs of toxicity. The integrity of tissues was analyzed after staining with Hematoxilin & Eosin (Magnification 10X).

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Parameters	Control	5 days	15 days
Glucose (mg/dL)	100.60 ± 22.1	134.87 ± 26.5	145.3 ± 27.1
Cholesterol (mg/dL)	57.64 ± 9.89	55.64 ± 14.6	56.45 ± 6.93
Triglycerides (mg/dL)	138.7 ± 34.9	131.85 ± 34.9	188.64 ± 33.7
Uric acid (mg/dL)	3.28 ± 1.14	3.55 ± 0.64	4.11 ± 0.50
Creatinine (mg/dL)	0.55 ± 0.15	0.58 ± 0.11	0.53 ± 0.14
Urea (mg/dL)	1.97 ± 0.4	1.92 ± 0.08	1.88 ± 0.09
AST (U/L)	195.3 ± 49.8	2015.1 ± 25.3	219.07 ± 29.27
ALT (U/L)	65.8 ± 7.56	63.81 ± 4.57	61.66 ± 9.62
ALP (U/L)	46.85 ± 9.8	51.23 ± 10.5	47.1 ± 11.9

Table 1: Biochemistry parameters analysed in male rats
Data are means ± S.D.
*p<0.05 as compared with control group

Parameters	Control	5 days	15 days
Glucose (mg/dL)	100.61 ± 20	119.72 ± 20	130.50 ± 29
Cholesterol (mg/dL)	57.66 ± 8.4	65.13 ± 6.7	73.28 ± 9.6
Triglycerides (mg/dL)	101.94 ± 32	210.35 ± 31*	217.85 ± 35.8*
Uric acid (mg/dL)	2.83 ± 0.74	2.58 ± 0.24	2.79 ± 0.35
Creatinine (mg/dL)	0.35 ± 0.07	0.38 ± 0.07	0.39 ± 0.05
Urea (mg/dL)	1.97 ± 0.42	2.02 ± 0.34	1.91 ± 0.19
AST (U/L)	141.62 ± 20	142.66 ± 17	148.41 ± 11
ALT (U/L)	48.83 ± 4.12	43.11 ± 1.41	49.93 ± 11
ALP (U/L)	30.00 ± 13.5	39.29 ± 6.4	37.21 ± 10.8

Table 2: Biochemistry parameters analyzed in female rats
Data are means ± S.D.
*p<0.05 as compared with control group

of quantum dot coated with certain sugar molecules might facilitate the attraction to receptors in specific tissues and organs [23].

However, the synthesis of each individual type of QD determines its own unique physicochemical properties, which in turn determines its potential toxicity or lack thereof [23,24]. In addition, the complexity of the biological environment in a complete organism means that nanoparticles observed *in vitro* conditions may yield entirely different effects *in vivo*. This study evaluates the biocompatibility of CdS-MDx QDs in male and female rodents. Previous reports by this group have shown that CdS-MDx QDs were able to induce cell death by apoptosis and necrosis, their embryo toxicity, in a dose dependent manner, was also observed in *in vitro* and *in vivo* conditions [13]. Our present results demonstrate that the administration of 200 µg/Kg CdS-MDx QDs to rodents during 5 or 15 days was biocompatible and non-toxic.

Although studies are limited, the distribution of QDs across tissue/organs seems to be multi factorial, depending on QD size, QD core-shell components, and the bioactivity of conjugated or otherwise attached functional groups [25]. Size alone can markedly affect distribution kinetics [26]. Present results show that, after intraperitoneal injection, CdS-MDx QDs were distributed across all studied tissues. This suggests that the size of CdS-MDx QDs allowed their access into tissues and, like in the *in vitro* assay, there was cellular uptake of QDs in all tissues. QDs lacking specialized functional groups or specificity have been shown to incorporate into a variety of cell types via endocytic mechanisms, both *in vivo* and *in vitro* conditions [27]. An interesting find was the detection of bright green emitting in brain and testis, meaning that CdS-MDx QDs were able to cross the blood brain barrier (BBB) and the blood testicular barrier. Although previous studies on the biodistribution of NPs demonstrated their presence in the brain [28,29], no detailed analyses have been conducted on how frequently QDs are introduced into this organ. However, present preliminary results offer a basis for the development of novel CdS-MDx QDs with targeting molecules and drugs, which could enhance drug delivery efficacy across the BBB and facilitate the uptake of the QD-drugs into the brain.

Although few *in vivo* studies exist, these suggest that QDs may accumulate in a variety of organs and tissues [30,31]. Nanoparticles are commonly sequestered by phagocytic monocytes and macrophages from spleen and liver [32], and most of the *in vivo* biodistribution studies of nanoparticles have shown the accumulation of QDs in liver, spleen and kidney [33]. Present results evidence CdS-MDx QDs are indeed present in those organs. However, the pattern of biodistribution and accumulation of CdS-MDx QDs in other organs was different, even considering exposure time. It has been reported that QD surface coating can govern serum lifetime and pattern of deposition *in vivo* [34,35]. Now it is clear that QD ADME properties and toxicity depend on multiple factors derived from both inherent physicochemical properties as well as environmental conditions. Currently, there are no studies comparing the effect of QDs in both sexes. Our findings did not reveal any differences in the CdS-MDx QDs' pattern of distribution in female and male rats.

Safety concerns regarding inorganic nanoparticles are a key factor in their clinical application. In fact, substantial *in vivo* studies have documented that QDs can pose several forms of toxicity, including nephrotoxicity, hepatic damage, reproductive toxicity and hematological abnormality [36,37]. Our histopahological analysis of stained tissues showed that CdS-MDx QDs did not produce morphological changes in the structure of any of the tissues analyzed, nor were they correlated to any kind of abnormality in the biochemical analysis. The increase in serum triglycerides levels in female rats could be due to sex-specific differences in lipid and glucose metabolism [38]. It has been reported that estrogens display a high free fatty acids uptake and induce a high rate of triglyceride synthesis in subcutaneous adipose tissue, as well as an increase in the serum levels of triglycerides [39,40]. However, additional studies are needed to clarify the effects of CdS-MDx QDs on lipid metabolism in females.

Conclusion

Present results demonstrated that, when administered to rodents, CdS-MDx QDs were biocompatible and non-toxic. The pattern of biodistribution and accumulation of CdS-MDx QDs was different for all organs and exposure times, but not differences between sexes were found. CdS-MDx QDs may warrant further evaluation because they appear to be promising nanomaterials that can be used in biomedicine for bioimaging.

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