

ZAIS-BASED COLLOIDAL QDS AS FLUORESCENT LABELS FOR THERANOSTICS: PHYSICAL PROPERTIES, BIODISTRIBUTION AND BIOCOMPATIBILITY

Istomina MS^{1,2}✉, Pechnikova NA^{1,3,4}, Korolev DV¹, Pochkayeva EI¹, Mazing DS², Galagudza MM^{1,5}, Moshnikov VA², Shlyakhto EV¹

¹ Institute of Experimental Medicine, Almazov National Medical Research Centre, St. Petersburg

² Department of Micro- and Nanoelectronics, St. Petersburg State Electrotechnical University "LETI", St. Petersburg

³ Center of Experimental Pharmacology, St. Petersburg State Chemical Pharmaceutical Academy, St. Petersburg

⁴ Laboratory of Parasitic Arthropods, Zoological Institute of Russian Academy of Sciences, St. Petersburg

⁵ Department of Pathophysiology, Pavlov First St. Petersburg State Medical University, St. Petersburg

In recent years there has been an increase in interest in the use of colloidal quantum dots (QDs) in biology and medicine. In particular, QDs can be a perspective nanoscale object for theranostics, in which due to the specific accumulation of drug-loaded QDs in the pathological focus, its simultaneous visualization and targeted therapeutic influence occur. One of the serious limitations of the use of QDs in medicine is their potential toxicity, especially when the nanocrystal material contains elements such as cadmium or plumbum. Therefore, it is promising to develop labels based on QDs of relatively less toxic semiconductors of group I-III-VI, such as CuInS_2 and AgInS_2 . In this study, biodistribution and biocompatibility of QDs based on the AgInS_2 compound with a ZnS shell (ZAIS) are considered. In the study of biodistribution, the accumulation of QDs in organs such as liver, lungs, heart and kidneys was revealed. It was shown that QDs in the dose range from $2 \cdot 10^{-7}$ to $4 \cdot 10^{-6}$ M/L at intravenous administration in rats does not have a significant effect on body mass dynamics and basic hematological parameters for 30 days. Thus, ZAIS QDs can be used to visualize tissues and organs in various pathological processes, and immobilization of the drugs on their surface will allow to approach their application for theranostics.

Keywords: colloidal quantum dots, QDs, ZnS-AgInS₂, ZAIS, theranostics, biodistribution, biocompatibility

✉ **Correspondence should be addressed:** Maria S. Istomina
Parkhomenko 15 B, St. Petersburg, 194156; istominams1993@mail.ru

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ИССЛЕДОВАНИЕ КОЛЛОИДНЫХ КВАНТОВЫХ ТОЧЕК $\text{AGINS}_2/\text{ZNS}$ В КАЧЕСТВЕ ФЛУОРЕСЦЕНТНЫХ МЕТОК ДЛЯ ТЕРАНОСТИКИ: ФИЗИЧЕСКИЕ СВОЙСТВА, БИОРАСПРЕДЕЛЕНИЕ И БИОСОВМЕСТИМОСТЬ

М. С. Истомина^{1,2}✉, Н. А. Печникова^{1,3,4}, Д. В. Королев¹, Е. И. Почкаева¹, Д. С. Мазинг², М. М. Галагудза^{1,5}, В. А. Мошников², Е. В. Шляхто¹

¹ Институт экспериментальной медицины ФГБУ «НМИЦ имени В. А. Алмазова», Санкт-Петербург

² Санкт-Петербургский государственный электротехнический университет «ЛЭТИ» имени В. И. Ульянова (Ленина), Санкт-Петербург

³ Центр экспериментальной фармакологии, Санкт-Петербургский государственный химико-фармацевтический университет, Санкт-Петербург

⁴ Зоологический институт РАН, Санкт-Петербург

⁵ Первый Санкт-Петербургский государственный медицинский университет имени И. П. Павлова, Санкт-Петербург

В последние годы отмечается повышение интереса к использованию коллоидных квантовых точек (КТ) в биологии и медицине. В частности, КТ могут представлять собой перспективные наноразмерные объекты для терагностики, при которой за счет специфического накопления нагруженных лекарственным соединением КТ в патологическом очаге происходят одновременно его визуализация и таргетное терапевтическое воздействие. Одним из ограничений использования КТ в медицине является их потенциальная токсичность, особенно если материал нанокристалла содержит такие элементы, как кадмий и свинец. В связи с этим перспективной представляется разработка меток на основе КТ относительно менее токсичных полупроводников группы I-III-VI, таких как CuInS_2 и AgInS_2 . Целью работы было исследование биораспределения и биосовместимости КТ на основе соединения AgInS_2 в оболочке ZnS. Для этого проводили синтез КТ инъекционным методом, изучали размеры получаемых КТ, их спектры поглощения и фотолюминисценции. Методом флуоресцентного имиджинга исследовали *in vivo* биораспределение КТ. Биосовместимость образцов определяли *in vivo* по динамике изменения массы тела животных и при помощи гематологических исследований. При изучении биораспределения было выявлено накопление КТ в таких органах, как печень, легкие, сердце и почки. Показано, что КТ в диапазоне доз от $2 \cdot 10^{-7}$ до $4 \cdot 10^{-6}$ моль/л при внутривенном введении крысам не оказывают значимого влияния на динамику массы тела и основные гематологические показатели на протяжении 30 дней. Таким образом, КТ на основе соединения AgInS_2 в оболочке ZnS могут быть использованы для визуализации тканей и органов при различных патологических процессах, а возможность иммобилизации на их поверхности лекарственных средств позволит рекомендовать их к применению для терагностики.

Ключевые слова: коллоидные квантовые точки, $\text{AgInS}_2/\text{ZnS}$, терагностика, биораспределение, биосовместимость

✉ **Для корреспонденции:** Мария Сергеевна Истомина
пр. Пархоменко, д. 15 «Б», г. Санкт-Петербург, 194156; istomina_ms@almazovcentre.ru

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Recently, a new approach to the development of pharmaceutical compositions has been actively developed, consisting in the simultaneous resolution of therapeutic and diagnostic problems [1]. For this purpose, various fluorophores can be used as diagnostic markers [2]. However, in world practice only two fluorophore are allowed for clinical use, indocyanine green and various combinations of fluorescein [3]. Besides, fluorescent dyes have a significant disadvantage, the ability to fade with time. A material in which there is no fading out are colloidal quantum dots (QDs) [4]. Besides, many QDs have toxic properties [5]. The main disadvantage of these systems is the toxicity of the crystal core of the material when used in biomedicine. Since in core-shell-like structures, the core is often a compound containing a heavy metal, the shell does not always cover the core or can be destroyed, which leads to the release of heavy metal ions into the body. Also, it was suggested that the toxicity of QDs can be correlated with the physicochemical properties of the shell, the nature of surface "ligands" (providing colloidal stability), the presence of other surface modifications and interactions with various molecules (e.g., proteins) present in biological environments [6–11]. Therefore, an important practical task is the development of non-toxic QDs and the study of their biocompatibility. The results of studies carried out in recent years decisively showed that modifying the surface of QDs or using QDs of a certain composition is accompanied by a significant increase in the biocompatibility of these objects. For example, ZnS-CdSe QDs conjugated with tripeptide arginine-glycine-aspartic acid (RGD) in systemic administration in mice showed no toxic properties in the histological study, and analysis of the tissue samples by mass spectrometry did not reveal Cd²⁺ ions [12]. In [13], a shell of biocompatible copolymers based on 2-(2-methoxyethoxy) ethyl methacrylate and oligo(ethylene glycol) methacrylate was grown on the surface of ZnO QDs by surface-initiated radical polymerization. Analysis of cytotoxicity to human colon cancer cells HT29 has revealed that QDs with polymer coating showed virtually no toxicity at concentrations up to 12.5 µg/mL, whereas when loaded with doxorubicin, high cytotoxicity and decreased viability of HT29 cells occur. In [14], nanocomposites based on silver selenide QDs with an average size of 11.4–12.7 nm, luminescing in the transparency region of biological tissues (705 nm), were obtained and characterized in detail. The absence of toxic properties of materials is achieved using the stabilizing potential of the galactomannan, a natural polysaccharide, as well as a simple, environmentally friendly way of generating highly reactive selenide anions acting as a selenium-containing agent. Carbon QDs and their combinations with various nanoparticles (e.g., based on iron [15]) also are non-toxic.

QDs that do not contain potentially toxic elements in their composition are of specific interest. Such QDs include, in particular, QDs based on the AgInS₂ compound in the ZnS

shell (ZAIS). In the present work, the physical properties, biodistribution and biocompatibility of ZAIS QDs were studied.

METHODS

Synthesis of colloidal quantum dots

The study objective in this research were ZAIS colloidal quantum dots. Chemical synthesis of QDs was carried out by injection method in an aqueous medium. To achieve a balance of the reactivity of indium and silver cations in the synthesis, ligands such as L-glutathione and sodium citrate were used. Precursors of silver nitrate, AgNO₃ (0.005 mM) and indium nitrate, In(NO₃)₃•4.5H₂O, (0.02 mM) were placed and dissolved in 5 mL of distilled water in a 10 mL flask. Subsequently, 0.01 mM of L-glutathione and 0.08 mM of sodium citrate (200 µL of an aqueous solution) were added to this solution. The anion precursor solution contains of 0.04 mM Na₂S•9H₂O in 500 µL of distilled water. The precursor solution of sulfur was injected into the initial solution at room temperature, then it was heated to 95 °C for 40 min by the flask heater. To create a shell consisting of zinc sulfide, 0.02 mM of zinc nitrate (Zn(NO₃)₂•6H₂O) and 0.02 mM of sodium sulfide (Na₂S•9H₂O) were dissolved in 200 µL of distilled water. After cooling of the initial solution of the nanocrystal cores to room temperature, a precursor solution of zinc nitrate and sodium sulfide was simultaneously added into it (drop by drop), then it was heated to 95 °C for 40 min. To isolate the particles from the initial solution, isopropyl alcohol was added followed by centrifugation.

Characterization of colloidal quantum dots

The size of the colloidal quantum dots was estimated by the method of dynamic light scattering, which can also be used to determine the profile of small particle size distribution in suspensions, emulsions, micelles, polymers, proteins, nanoparticles or colloids, by laser particle size analyzer SZ-100 (Horiba Jobin Yvon, Kyoto; Japan) with a range of nanoparticle diameters measuring from 0.3 nm to 8 µm.

The optical absorption spectra of the samples were measured by a spectrophotometer PE-5400UV (ECROSKHIM Co., Ltd.; Russia), and the photoluminescence spectra were obtained by a specially developed spectrofluorometer based on the monochromator MDR-206 (Lomo Fotonika; Russia).

Estimation of QDs biodistribution

Fluorescent imaging of biological samples was carried out on optical imaging system IVIS Lumina LT Series III (PerkinElmer; U.S.A.). After preliminary studies of the absorption and photoluminescence spectra, the filters were optimally matched for ZAIS QDs. The excitation wavelength for these

Table 1. Groups of animals and concentrations of injectants for the study of QDs biocompatibility

Group designation	Injectant	Concentration of injectant, M/L	Dose of the active substance, ml	Time of the experiment, days	Number of animals in the group
QDs-L(15)	QDs	4 • 10 ⁻⁶	1	15	5
QDs-M(15)	QDs	2 • 10 ⁻⁶	1	15	5
QDs-S(15)	QDs	2 • 10 ⁻⁷	1	15	5
Control	NaCl	–	1	30	5
QDs-L(30)	QDs	4 • 10 ⁻⁶	1	30	5
QDs-M(30)	QDs	2 • 10 ⁻⁶	1	30	5
QDs-S(30)	QDs	2 • 10 ⁻⁷	1	30	5

QDs was $535 \text{ nm} \pm 20 \text{ nm}$, the emission wavelength was $655 \text{ nm} \pm 20 \text{ nm}$.

Study of QDs biocompatibility

Biocompatibility assessment was carried out on SPF Wistar male rats (Nursery of laboratory animals "Pushchino"). The body weight of the animals was $235 \pm 10\%$. The tested QDs were injected into the lateral tail vein for 3 min. The formation of groups of animals and their brief characteristics are presented in Table 1. To identify the QDs and their concentrations, the following notations were introduced: the QDs concentration of $3.7 \cdot 10^{-9} \text{ M/kg}$ — L (large), $1.85 \cdot 10^{-9} \text{ M/kg}$ — M (medium), $1.85 \cdot 10^{-10} \text{ M/kg}$ — S (small). At 15 and 30 days after intravenous administration of QDs, hematological parameters, body mass dynamics were recorded in animals, and animal death was also taken into account.

Hematologic studies

Hematologic studies were performed using the hematology analyzer URIT-3000 Vet Plus (URIT Medical Electronic; China). To assess the influence of QDs on the body, the following hematologic parameters were studied: red blood cells (RBC), mean corpuscular volume (MCV), white blood cells (WBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean platelet volume (MPV), hematocrit (HCT), platelets (PLT).

Statistical analysis

Testing the hypothesis on the equality of average sample sizes in several dependent samples was carried out by the methods of the variance analysis for repeated measurements; the values in the groups was analyzed by nonparametric statistical methods using median (50th percentile) and interquartile range (IQR; 25th to 75th percentile). Testing the hypothesis on the equality of average sample sizes in independent samples was carried out using the Mann–Whitney test. Differences at a significance level of $p < 0.05$ were considered statistically significant. The calculations were performed using the software GraphPad Prism 7.04 (GraphPad Software Inc.; U.S.A.).

RESULTS

Characteristics of the ZAIS QDs

Study of QDs by the dynamic light scattering method is shown in Fig. 1. According to the study, the largest proportion of QDs had an average radius of 3 to 4.5 nm.

Extinction and photoluminescence spectra of aqueous dispersion of the ZAIS QDs are shown on Fig. 2. The QDs dispersion showed an emission peak at 627 nm.

The photoluminescence spectrum is distinguished by a noticeable asymmetry and a rather large half-width at half-height. Together with a large Stokes shift, this indicates the mechanism of photoluminescence due to defects, internal and, possibly, surface [16–19]. In this case, the half-width of the

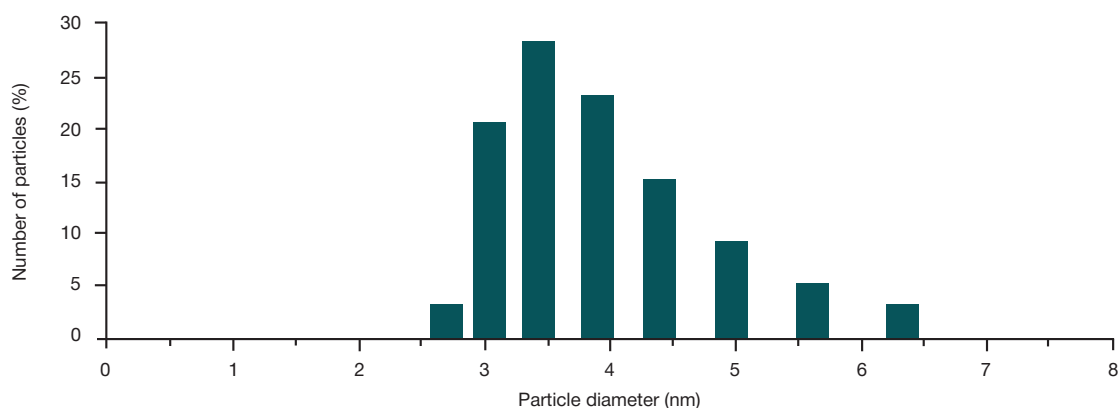


Fig. 1. Histogram of the diameter distribution of the ZAIS QDs obtained in the dynamic light scattering study

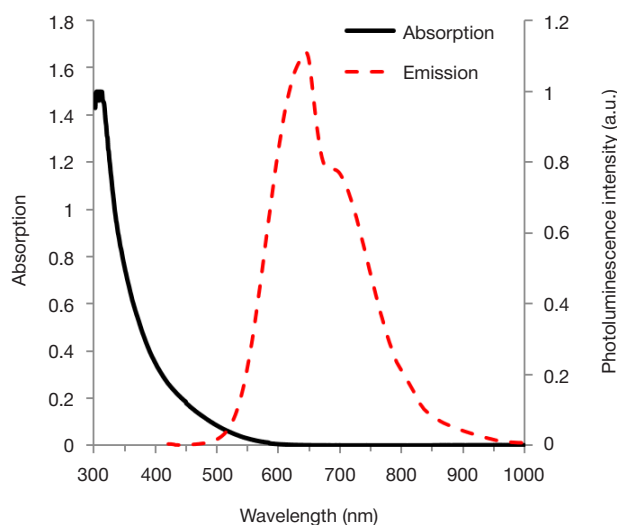


Fig. 2. Absorption and photoluminescence spectra of ZAIS nanocrystals in distilled water

spectrum can depend not only on the particle size distribution, but also on the distribution and nature of the defects in nanocrystals [20]. The absorption spectrum does not contain pronounced inflection points or maxima, which is typical for nanocrystals of triple metal chalcogenides [16, 21].

QDs biodistribution

Preliminary assessment of QDs biodistribution in *ex vivo* organs was performed at 1 and 24 hours after intravenous QDs administration at a dose of $4 \cdot 10^{-6}$ M/L by an optical imaging system IVIS Lumina LT Series III (PerkinElmer; U.S.A.) (Fig. 3).

In the study of biodistribution of ZAIS QDs, an accumulation of nanoparticles in time was noted in such organs as liver, kidneys, lungs and heart. The liver fluorescence intensity at 24 hours after the QDs administration was significantly higher than at 1 hour after administration, which indicates the QDs accumulation in the liver during the first 24 hours after administration, whereas significant differences in fluorescence levels in other organs at 1 hour and 24 hours was not noted.

Body weight of animals

The dynamics of body weight in animals of all experimental groups is shown in Fig. 4.

Statistical analysis of the data showed no significant differences in the body weight of animals in the experimental groups compared with the control throughout the entire experiment ($p > 0.05$).

Hematological parameters

Main hematological parameters, measured on days 15 and 30 after the QDs introduction, are shown in Fig. 5.

Changes in hematological parameters of experimental groups did not show a significant difference in comparison with the control group ($p > 0.05$).

DISCUSSION

QDs are an excellent alternative to traditional organic fluorophores because their size, surface chemistry, spectral properties and stability can be easily adjusted to optimize *in vivo/in vitro* imaging. The colloidal QDs, synthesized by the injection method in an aqueous medium, were used for *ex vivo* imaging. At the moment, QDs are being developed and used in biomedicine for various purposes, such as drug delivery, diagnostic procedures, tumor visualization [21–30]. It should be noted that the problems of QDs biodistribution are currently being studied extensively, in particular, according to the publications of foreign authors, their use shows a positive result as cell markers for imaging tumors of different tissues [31–36]. In this case, the main target organs, in which QDs accumulate, are liver, kidneys and spleen [37–39], as well as lungs [40], skin, gastrointestinal tract and bladder [41], besides these QDs were found in lymph nodes [42]. Our data suggest that ZAIS QDs have a significant tropism to the liver, as evidenced by fluorescence intensity increase within 1 to 24 hours after intravenous administration. In addition, QDs in the dose range from $2 \cdot 10^{-7}$ to $4 \cdot 10^{-6}$ M/L did not have systemic toxicity, which is confirmed by the absence of significant changes in body mass dynamics and significant differences in hematological parameters, absence of animal death within 30 days after administration. Taking into account the fact that in most cases the experimental samples of ZnS-Cd/Se QDs [43] have pronounced systemic toxicity, which affects, in particular, hematological parameters [44], it can be assumed that ZAIS QDs after additional testing on animals can be used as fluorophores in medical practice, and immobilization of the drugs on their surface will allow to approach their application for theranostics.

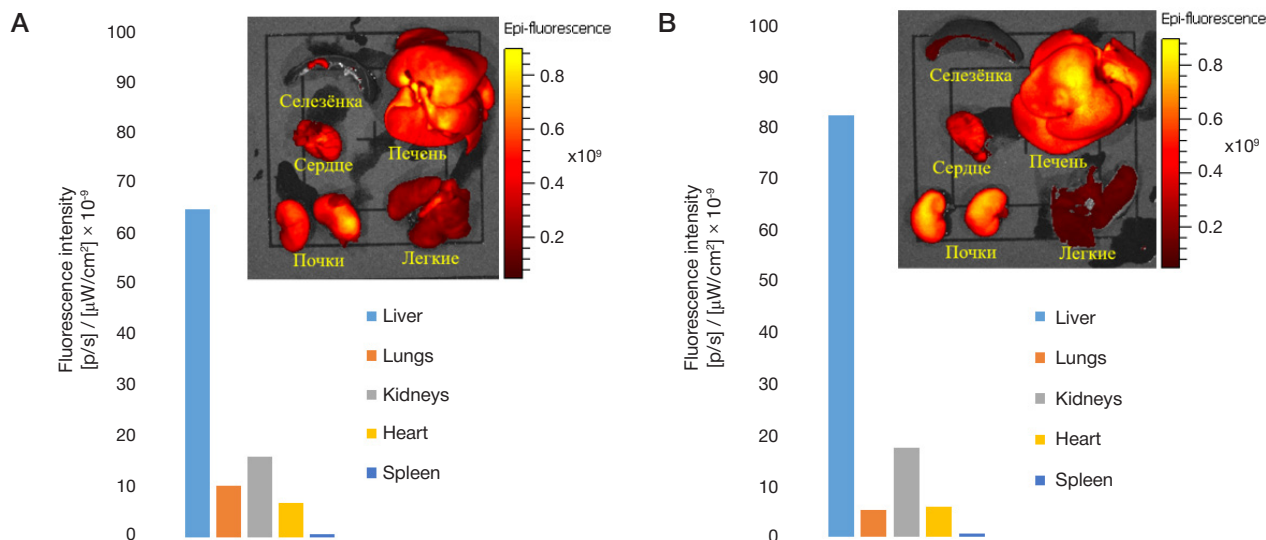


Fig. 3. Visualization of the intensity of fluorescent radiation in the rat organs at 1 hour (A) and 24 hours (B) after the ZAIS QDs administration

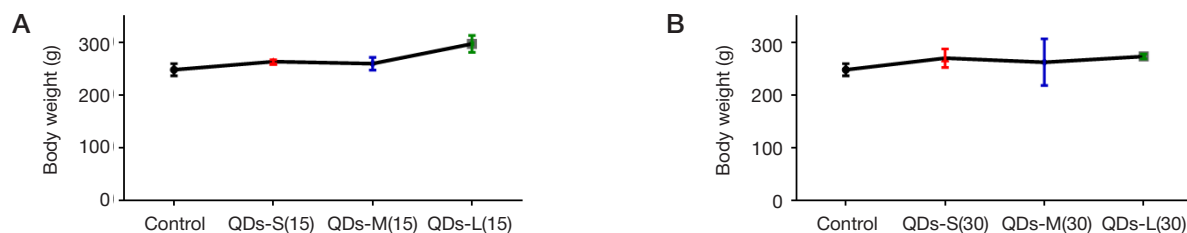


Fig. 4. Animal body weight in the animals 15 days after QDs administration (A) and 30 days after QDs administration (B)

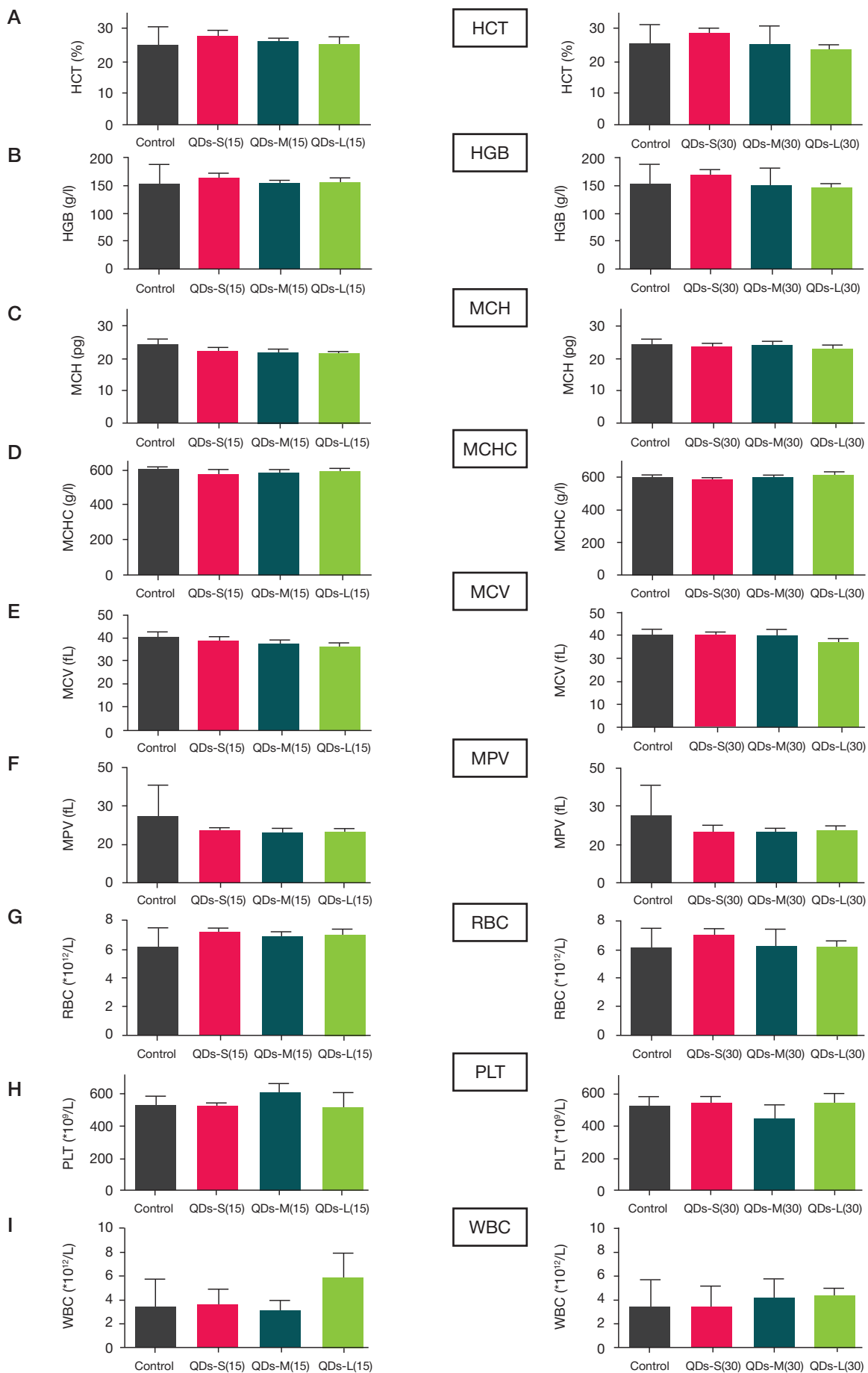


Fig. 5. Results of the research of hematological parameters in the control and at 15 and 30 days after QDs intravenous administration at various doses

CONCLUSIONS

Bioluminescence research of colloidal quantum dots obtained by the injection method in the aqueous medium, demonstrated them as stable agents that can be used in long-term studies.

Biodistribution evaluation shows that these ZAIS QDs have a tendency of accumulating in organs such as liver, lungs, heart and kidneys. A study of the toxic effect on hematologic parameters and body mass dynamics showed that these QDs have no pronounced toxicity.

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