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Quantum dots mediated embryotoxicity via placental damage

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ABSTRACT

The increasing use of nanoparticles in consumer products raises the concerns of their safety. This study investigated the biological effects of quantum dots (QD) exposure to rats during pregnancy. CdTe QD were injected on the 13th gestation day. Morphological features of 121 fetuses and histological analysis of placentas were performed on the 20th gestation day. The results showed that QD exhibit dose dependent embryotoxicity: survival rates of fetuses were 97% (5 mg/kg dose), 86% (10 mg/kg dose) and 43% (20 mg/kg dose). QD exposure also resulted in the reduction of fetal body length and mass, disturbed ossification of limbs and caused placental tissue damage. QD exhibit no teratogenic effects at the applied doses. It is hypothesized that embryogenesis was impeded due to the placental damage rather than QD penetration and accumulation in the fetuses. To conclude, mothers should be protected from QD exposure during pregnancy.

1. Introduction

With the rapid development of nanotechnologies and increasing application of nanoparticles (NP) in consumer and medical products, new questions are consistently being raised regarding their safety and possible adverse effects on human health. Commonly, NP are defined as materials in the size range of 1–100 nm which is comparable to intercellular pores of the biological barriers. Therefore, extensive knowledge about the factors and mechanisms of NP penetration through biological barriers is essential for their safe application.

Pregnancy is the most important and sensitive period of human development, as the fetus is highly susceptible to chemical exposure. Since many classical medical procedures may not be applied during this period, NP such as quantum dots (QD) could be utilized as agents for diagnostics and treatment, in view of their penetration through biological barriers being more restricted than that of common organic drugs. Furthermore, owing to the unique optical properties of QD such as broad absorption spectra, high quantum yield, long lasting photostability, and efficient resonance energy transfer to the photosensitizer molecules, QD have the potential to be effectively applied in fluorescence imaging and photodynamic therapy (PDT) of cancer [1,2]. Considered to be one of the least invasive and safest cancer treatment methods [3], in some cases of pregnancy PDT is in fact the only possible therapeutic option available [4].

A human perfusion model revealed that syncytiotrophoblast cells are the key players in regulating NP transport across the human placenta. The main mechanism underlying this translocation is based not on passive diffusion, but is also likely to involve an active, energy-dependent transport pathway [5]. Besides, it was shown that transplacental passage depends on the stage of embryogenesis and that the barrier might effectively protect fetus from passage of NP when placenta is fully formed [6]. NP have also been shown to cross the placental barrier, but until now a more complete picture of the effect of NP on pregnant animals has been lacking. According to Sun J. et al., a great number of NP are transferred to the fetus through blood circulation, leading to the production of reactive oxygen species and consequent inflammatory processes [7]. Chu M. et al. showed that QD composed of CdTe may be transferred from pregnant mice to their fetuses across the placental barrier. Importantly, it was shown that the transfer rate depends on the size, superficial coating, and the dose of the QD [8]. Size dependent

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penetration was also reported for silica NP with the varying diameter of 70–1000 nm [9] and for gold NP from 1.4 to 18 nm in size, showing that smaller particles penetrate through the barrier easier [10]. Additionally, silver NP have the potential to disrupt fetal and postnatal health through epigenetic changes in the fetus and abnormal development of the placenta [11]. Whereas, polystyrene NP might be taken up by placental tissue and induce trophoblast cell apoptosis [12]. Biological interaction of solid NP first of all depends on their geometrical and superficial properties like size, polarity, biological affinity and colloidal stability [13,14]. The internal composition of the NP is of secondary importance and plays only a minor role, if any, in the interaction with the surrounding biomolecules. Therefore, the localization and biological effects of NP made from different materials, but possessing the same geometrical features and superficial chemistry, are reported to be very similar. Nevertheless, it is yet to be determined how safe it is to use NP containing products during pregnancy. In addition, the complete understanding of NP behaviour *in vivo* is pivotal for NP toxicological standardization.

Semiconductor QD serve as a perfect model for investigation of NP distribution and migration pathways *in vivo*, due to their bright and size-tunable photoluminescence and easy surface modification to achieve desired superficial properties. Many investigations have pursued an ultimate vision of using QD in clinical settings [15], however a great deal of concern was raised about the potential hazards of QD because of their heavy-metal content [16], which can cause phototoxic and teratogenic damage even at very low concentrations [17,18]. Several studies have argued that QD are not toxic when their surface is fully passivated with proper organic coatings [19,20]. Hauck T. S. et al. reported that no abnormal behaviour or tissue damage was noticed in mice and rats over periods of months after the systemic administration of QD [21].

Two main reasons are considered to cause QD toxicity: first, penetration of QD through the placental barrier, and second, their chemical stability in maternal organism [22]. QD might be retained by placental trophoblast cells, hence obstructing their ability to reach the fetus. In this case, QD cannot directly affect fetal development, however, heavy metals or other products of possible QD degradation can be transferred across the placenta and influence the formation of fetal organs [23,24].

With respect to the potential toxicity of QD compounds and the lack of knowledge about QD toxicity mechanisms, the purpose of this study was to assess the biological effects of QD exposure during pregnancy. The experiments aim to test the hypothesis whether QD can impede embryogenesis after passage to the maternal organism when the placental barrier is fully formed. Hence, the NP penetration through the placental barrier, effects on embryogenesis, and their safe application during pregnancy are herein investigated and discussed.

2. Materials and methods

2.1. Animals and treatment schedule

The study of the QD effects on fetal formation was carried out using 158 fetuses (33 in the control group). Albino *Wistar* rats (10–11 weeks old) were obtained from the State Scientific Research Institute of the Innovative Medical Center (Vilnius, Lithuania). The main characteristics of the animals are listed in Table 1. The strain is well documented and has a long research history, therefore, it was decided to use the most common strain to reveal the general principles of NP effects on embryogenesis.

The animal husbandry and experiments on animals were carried out according to the National and European regulations and were approved by the Lithuanian Animal Care and Use Committee (permission no. 0190).

Animals were kept under conditions of permanent temperature and humidity, and a standard light/dark cycle was maintained. Food and fresh drinking water were available *ad libitum*. After being acclimated for at least 7 days, female rats were mated overnight with males of the same strain. Vaginal smears from each female rat were collected and subjected to microscopic examination on the following morning in order to determine the estrous cycle and the presence of sperm. The day of sperm detection in vaginal smears was designated as gestation day (GD) 0.

2.2. Embryotoxicity and teratogenicity analysis

CdTe core QD were supplied in powder form by PlasmaChem GmbH, Germany. The surface of these particles is passivated with mercaptopropionic acid (MPA), which is attached to the CdTe core via thiol group, and the carboxyl group is exposed to the outside giving the negative charge to the QD.

Without further purification QD were dissolved in 0.5 ml saline at three doses for the experiments: 5 mg/kg, 10 mg/kg and 20 mg/kg. The solutions reached the concentrations of 10 mg/ml, 20 mg/ml, 40 mg/ml of CdTe QD, respectively. The 5–20 mg/kg doses were chosen according to the previous report of Chu M. et al., who reported adverse effects of QD on embryogenesis [8]. QD were injected into the pregnant rats intraperitoneally on the GD 13. The rats of the control group were injected with saline. One pregnant rat was injected with 100 mg/kg dose on the GD 13, but on the next day it started bleeding from vagina, indicating a miscarriage, and died.

All rats were subjected to the Caesarean section in the state of spinal cord dislocation on the GD 20. The uteruses were excised and the number of dead and living fetuses in the uterine were recorded to determine the post-implantation mortality indices. The fetuses, as well as the placentas were weighed.

The body length of the fetuses were measured and then fixated in the Buen's solution for detection of the external malformations, such as abnormal formation of organs, missing limbs, developmental defects, hernia, hydrocephaly, etc. After external examination, the fetuses were cut into slices and the visceral abnormality was analyzed with a stereomicroscope (set size of 60 fetuses).

In order to render the skeleton visible, we used the clarification method described before [25]. Briefly, the fetuses were fixated in ethanol solution and the soft tissues were macerated using caustic soda, then stained with alizarin red and cleared with glycerine. The lengths of the ossification centers of the stained fetuses' limb bones were measured in the middle of the diaphysis of the long tubular bones (maximum distance between proximal and distal epiphysis of joint surfaces) using an eyepiece micrometer attached to a stereomicroscope MBS-1 [26].

2.3. Histology of placenta

The uteri were dissected to collect the fetuses and placentas, which were then separated, weighed, and fixed in 10% buffered formalin for at least 24 h. The sections of placenta were paraffin-embedded, after which the tissue blocks were cut in serial sections of 3 μm, and stained with hematoxylin and eosin (H&E). Digital images were captured using the AperioScanScope XT Slide Scanner (Aperio Technologies, Vista, CA, USA) under 20× objective magnification. The visual evaluation was performed by a pathologist by analyzing virtual sample. The AperioImageScope software was used to quantify the thickness of placental layers.