INTRAVENOUSLY INJECTED CDTE QUANTUM DOTS EXCRETED THROUGH ENTEROHEPATIC PATHWAY AND CAUSEDGUTTOXICITY

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Abstract - CdTe quantum dots have great potential in clinical diagnostic imaging applications because of their excellent fluorescence properties.Because it contains the heavy metal cadmium, its toxicity to the human body has been concerned. However, few studies have investigated its possible toxicity based on its distribution and excretion behavior in the body. In this study, 12.5nmol/g quantum dots were injected intravenously into mice to observe their distribution and excretion behavior in vivo, followingexplored their possible toxic effects on intestine. The traditional view is that exogenous substances injected intravenously are excreted mainly through urine, but our results surprisingly found that quantum dots were excreted mainly through the enterohepatic pathway due to their increased size in vivo and caused toxic damage to the intestine. This study indicates the unique excretion behavior and thepotentialtoxictarget organ of gutfor quantum dots.

Keywords - Nanotoxicity; Gut toxicity; ADME; CdTe quantum dots; Excretion

I. INTRODUCTION

Quantum dots (QDs) are nanocrystals with a particle size of less than 10 nm, which are mainly used in biological imaging and advanced clinical diagnostic techniques.Cadmium telluride (CdTe) is the most commonly used QD because itsbest imaging effective.However, because it is a nanomaterial containing cadmium, its biological process and toxicity in vivo have attracted much attention.CdTeQD is mainly used in the clinic through intravenous injection, so its distribution, metabolism and excretion pathway directly determine its possible toxic target organs.Studies have shown that when the size of intravenously injected nanoparticles is greater than 15 nm, renal excretion is blocked while mainlyexcreted from the enterohepatic pathway[1].Although the original particle size of CdTeQD is less than 10 nm, the particle size of them may be larger after being wrapped by protein corona in vivo, so its metabolic pathway may be special. In this study, we artificially synthesized CdTe quantum dots coated with mercaptopropionic acid (MPA), monitored the way of excreting quantum dots after intravenous injection into mice, and explored the possible toxic damage. This study is ofsignificanceto the research of bio-metabolic pathways and toxicity of CdTe quantum dots in vivo.

II. METHODS

2.1 Synthesis of CdTe QDs

CdTe quantum dots were prepared by electrochemical method following our recent research[2].First, an ultra-pure water suspension of $CdCl_2$ and MPA was prepared and the pH was adjusted to 10.Then the CdTe precursor was prepared by electrochemical method in an electrochemical workstation under the condition of ice bath and N_2 flow.After reaction, a water bath at 80°C for 20 h allowed the CdTe crystals to grow to a suitable size.

2.2. Characterization

The morphology of the quantum dots was characterized by high-resolution transmission electron microscopy (HRTEM, Thermo Fisher,Talos F200X, 200 kV).Fluorescence spectrophotometer (HITACHI, F4600) was used to characterize the fluorescence luminescence properties of the quantum dots.The hydrodynamic diameters of the quantum dots in saline solution and cell medium containing serum were characterized by Marvin Zetasizer Nano ZS90.

2.3. Animalexperiment

20 ICR mice (18-22 g)were randomly assigned to control, 12 h, 24 h and 7 d groups, with 5 mice in each group.The animal experiments complied with the ARRIVE guidelines and were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and the guidelines have been followed.After one week ofacclimation, 12.5nmol/g quantum dots were injected through the tail vein.Our previous in vivo imaging studies have shown that the optimal imaging time of CdTe quantum dots in mice was 3hand the bestdose was 12.5nmol/g, so this study used this dose[3].The quantum dots werewashed to remove impurities before injection. The quantum dots were mixed with acetone (1:2), centrifuged at 15000 rpm for 20 min, then the supernatant was abandoned and re-suspended with normal saline.The control group was injected with the same amount of normal saline.

2.4. Excrements and organs collection

3 mice of the 7 d group were placed in metabolic

cages and their urine and feces were collected at 0 h, 8 h, 16 h, 24 h, 32 h and 44 h. Liver, kidney and small intestine tissues were collected from mice in control, 12 h, 24 h and 7 d group after euthanasia.

2.4. Cadmium in the organs and excreta of mice was detected by ICP-MS

The collected tissues and excreta were weighed and dissolved with concentrated nitric acid for 2 d, and 3:1 hydrogen peroxide was added and heated on the graphite electric heating plate at 200 ℃ .Then sampleswere dissolved with 1% dilute nitric acid and the volume was fixed to 4 mL. The Cd element was detected by ICP-MS after filtration with a0.22μm filter.

2.5. Hematoxylin-eosin staining

Jejunum tissues of mice in control group, 12 h group, 24 h group and 7 d group were intercepted, fixed with 4% paraformaldehyde for 24 h, dehydrated, paraffin embedded, and sliced. The slices were stained with hematoxylin and eosin, then sealed and observed under the microscope (Zeiss,Vert.A1).

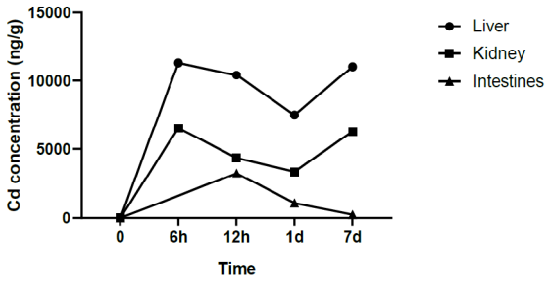


Figure 3 Distribution of cadmium in liver, kidney and intestine of mice treated with CdTe quantum dots for 6 h, 12 h, 1 d and 7 d

III. RESULTS

3.1. Characterization of CdTe QD

As shown in Figure 1, the lattice structure of the quantum dots prepared was obvious, and the primary particle size was about 5 nm. The 3d scanning contour map of fluorescence emission spectra also shows that CdTe QD exhibited the best fluorescence emission performance around 590 nm under 375 nm excitation, which proves its effectiveness.

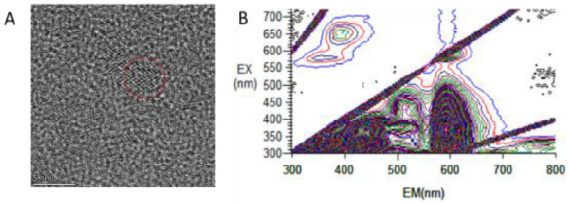


Figure 1Characterization of quantum dotsA. High-resolution transmission electron microscopy; B. Fluorescence emission Spectrum (3d scanning)

After testing the hydrodynamic diameters, we found that the hydrodynamic diameterof the quantum dot in the saline solution was about 6 nm, but after contact with the medium containing protein, the particle size increased to 32 nm(Figure 2), indicating that the protein corona envelope had increased its secondary particle size.

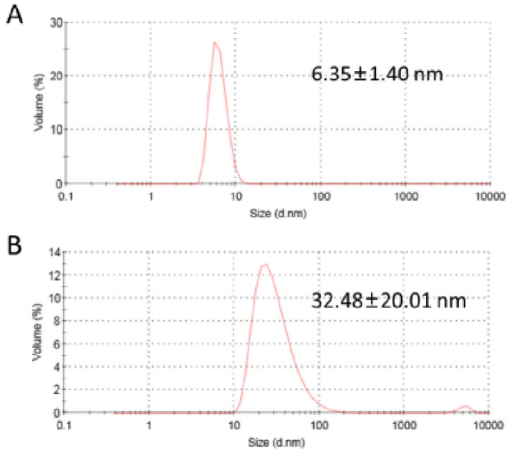


Figure 2Hydrodynamic diameters of CdTe quantum dots in normal saline (A) and complete culture (B)

3.1. Distribution of CdTe quantum dots in major organs

After detecting the distribution of quantum dots in the main organs of mice during different periods of exposure, we found that the liver was the highest accumulating organ, followed by the kidney. However, in addition to the main storage organs of the liver and kidney, the intestinal tract wasalso distributedby quantum dots within 7 d, which is not quite the same as the general distribution pathway of intravenous injection.

3.2. CdTe quantum dots excreted mainly in feces

We further monitored the amount of Cd in urine and feces collected at each time period.Unexpectedly, the presence of Cd element was almost all detected in feces rather than urine after intravenous injection of quantum dots.The excreted feces contained the highest content of Cd during the period of 8 h-12 h. The peak of fecal excretion was within 24 h.

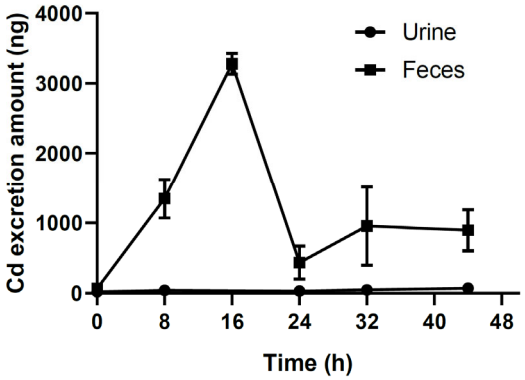


Figure 4Excretion of cadmium at different time periods after CdTe quantum dot treatment of mice

3.3. CdTe quantum dots may cause intestinal damage

Due to the unique excretion pathway of quantum dots, whether it will lead to intestinal damage after liver and intestine metabolism is worthy of further investigation. Our results show that quantum dots can lead to harmful effects in the gut, and may change over time. HE staining showed that at 12 h, the villi length of the small intestine became shorter and lamina propria vacuoles were increased. Crypt hyperplasia resulted the length ratio of villi crypt decreased.At 24 h, the villi were swollen and some were serrated. After 7 d, the villi recovered slightly, but some villi were still serrated, and goblet cells increased significantly.

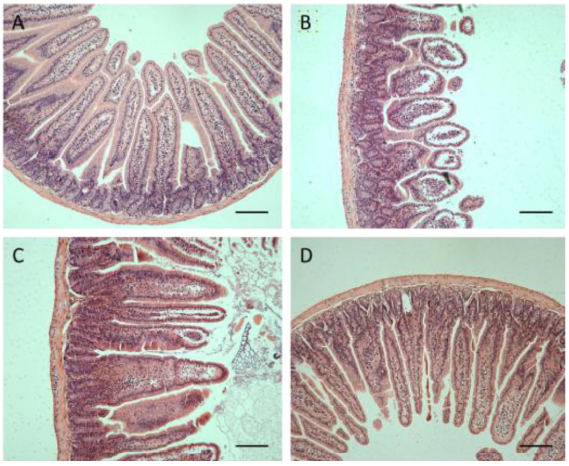


Figure 5 HE pathological photos of jejunum treated by CdTe quantum dots after 12 h (B), 24 h (C) and 7 d (D). Scale bar =100 μm, Magnification=100

IV. DISCUSSION

CdTe quantum dots are the most widely used quantum dots.Because it is mainly used in clinical diagnosis and directlycontact with the human body through intravenousinjection, its safety is concerned.In toxicology, absorption, distribution, excretion and metabolism (ADME) are important to study the dynamic changes of exogenous substancein vivo and to predict their biological effects.Therefore, the studyof ADME can determine the main target organs of exogenous substanceand provide important basis for targeted prevention and treatment.

It is generally believed that the metabolic pathway of excretion of intravenous drugs is often excreted rapidly through renal filtration along with urine.However, nanoparticles may exhibit unique metabolic excretion patterns due to their unique size effects.We monitored the distribution and excretion behavior of CdTe in the body by detecting the element Cd.Our results suggest that feces are the primary pathway for the excretion of CdTe quantum dots, rather than urine.Consistent with the results from Misra et al., nanoparticles form protein corona in the biofluid, resulting in a large increase in particle size and thus exceeding the limit of renal filtration[1].In the biological medium, the hydrodynamic diameter of CdTe has reached 32 nm, which is far beyond the limit of glomerular filtration.Yang et al. also showed that feces are the main excretion pathway for ZnS and ZnO quantum dotsby intravenous injection[4].Possible ways in which intravenously injected nanoparticles are excreted in feces are:1) enterohepaticpathway.After the accumulation and metabolism of the liver, CdTe is discharged into the duodenum through the bile duct[5];2)penetrating the gut capillaries into the intestinal tissue.

The unique excretion pattern of CdTe quantum dots indicates that intestinal tissue may be one of the potential important target organs that has been neglected.The intestine is an important organ for nutrient absorption and physiologic barrier.Therefore, the toxic effect on the gut may lead to the destruction of the body's digestion and absorption functions and defense functions[6].Our results show that the continuous excretion of quantum dots during 7 d leaded to significant pathological changes in jejunum tissue. During the acute phase, intestinal villi became shorter and swollen, and the length ratio of villus/cryptdecreased.The increase of goblet cells was the main result at 7 d.The intestinal villi edge can secrete a variety of digestive enzymes, so the longer the villi, the stronger the digestion and absorption capacity.The depth of crypt determines the rate of epithelial cell formation in intestinal villi mitosis, and reflects the cell formation rate, while the shallow crypt indicates the increase of cell maturation rate and the enhancement of secretion function.Therefore, the ratio of villus height to crypt depth can comprehensively reflect the digestive and absorption function of small intestine.The decrease of this ratio after CdTe treatment indicates that the mucosa may be damaged and the digestion and absorption capacity may bereduced[7].Goblet cells can secrete and release mucin. The increase of goblet cells may indicate the defense response of tissues against CdTe quantum dots[8].

V. CONCLUSION

In conclusion, our results show that the intravenously injected CdTe quantum dots are excreted mainly through the enterohepatic pathway due to the enlarged size in vivo beyond the renal excretion range.This may further lead to toxic effects on intestinal tissue.This conclusion provides important information for further understanding of the biological effects of quantum dots in vivo.

REFERENCE

[1] Misra P, Itty Ipe B, Tanaka E, et al. Renal clearance of quantum dots[J]. Nature Biotechnology. 2007, 25(10): 1165-1170.

[2] Liu N, Liang Y, Wei T, et al. The role of ferroptosis mediated by NRF2/ERK-regulated ferritinophagy in CdTe

QDs-induced inflammation in macrophage[J]. Journal of Hazardous Materials. 2022, 436: 129043.

[3] Wu T, He K, Liang X, et al. The glycolytic shift was involved in CdTe/ZnS quantum dots inducing microglial activation mediated through the mTOR signaling pathway[J]. Journal of Applied Toxicology. 2019.

[4] Yang Y, Lan J, Xu Z, et al. Toxicity and biodistribution of aqueous synthesized ZnS and ZnO quantum dots in mice[J]. Nanotoxicology. 2012, 8(1): 107-116.

[5] Poon W, Zhang Y, Ouyang B, et al. Elimination Pathways of Nanoparticles[J]. ACS Nano. 2019, 13(5): 5785-5798.

[6] Huang X, Tang M. Review of gut nanotoxicology in mammals: Exposure, transformation, distribution and toxicity[J]. Science of The Total Environment. 2021, 773: 145078.

[7] Chen Y, Lin Y, Nagy T, et al. Subchronic exposure to cellulose nanofibrils induces nutritional risk by non-specifically reducing the intestinal absorption[J]. CARBOHYDRATE POLYMERS. 2020, 229(115536).

[8] Turner J R. Intestinal mucosal barrier function in health and disease[J]. Nature Reviews Immunology. 2009, 9(11): 799-809.

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