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Quantum dots-hemin: Preparation and application in the absorption of heme iron

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Abstract

The absorption mechanism of heme iron remains unclear due to the limit of labeling techniques. Quantum dots (QDs) are powerful fluorescent probes resistant to photobleaching, however, there is no data about the application of QDs in heme iron absorption. Herein, we prepared hemin-coated CdSe/ZnS (QDs-hemin), and study their absorption *in vitro* and *in vivo*. Results showed that QDs-hemin had uniform particle sizes, physiological stability and high joint efficiency. Moreover, QDs-hemin could be successfully absorbed gradually into the duodenum with the time using synchrotron radiation micro X-ray fluorescence and confocal laser scanning microscopy. Furthermore, QDs-hemin were observed to degrade in lysosomes, and their absorption was blocked by Heme Carrier Protein 1 (HCP1) antibody and HCP1 siRNA. All the results demonstrate that QDs can be a good tracer for heme iron and that HCP1 pathway is critical and predominant over the endocytosis pathway in the absorption mechanism.

Key words: Heme; Quantum dots; Heme carrier protein 1; Micro X-ray fluorescence; Intestine

Heme iron and non-heme iron are two fundamental forms of iron in the body, and approximately 70% of the total iron exists in the form of heme iron (ferrous protoporphyrin IX), such as in hemoglobin, myoglobin, cytochromes and neuroglobin.[1,2] Iron deficiency can cause anemia and other bad effects, and supplementation of heme iron is the first choice to treat anemia. However, the absorption and regulatory pathways of heme iron have not been fairly well-studied although it has been known for a long period.

In 1955, heme-derived iron was first reported to be absorbed by enterocytes.[3] More than half-century later, the absorption of heme iron has yet to be identified. Some reports state that heme is absorbed by active transport hemoglobin carrier protein-1 (HCP1),[4] but others with an opposing view.[5,6] In addition, other proposed uptake pathways of heme iron add the complexity to understand its absorption.[7]

The lack of effective markers and labeling methods greatly limit the research progress on heme iron. Traditionally, heme is labeled with ^{55}Fe, ^{59}Fe and ^{14}C to trace iron and protoporphyrin IX, respectively, in the absorption and metabolic processes.[8] However, radiotracers cannot mark Fe and protoporphyrin simultaneously and in real time,[9] they cannot indicate whether heme is absorbed as an intact metalloporphyrin or as free iron and where it is degraded, either. Therefore, a more efficient method for tracking heme absorption is urgently needed to facilitate investigations on the predominant mechanistic pathway.

Conflict of interest: All the authors declare that there are no conflicts of interest.

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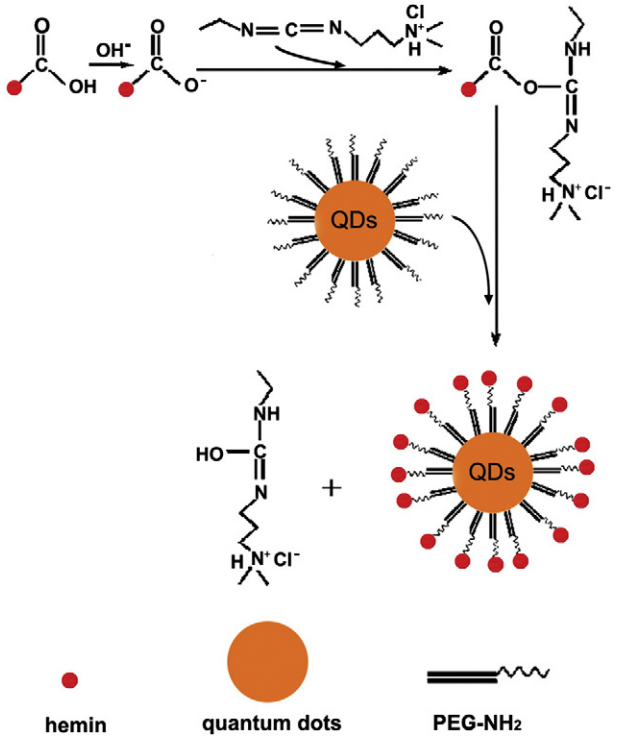

Figure 1. Graphic representation of preparative reactions for QDs-hemin.

Surface-modified quantum dots (QDs) have been extensively investigated as fluorescent probes for sensing and biosensing events, and as a class of agents for biomedical imaging and diagnosis due to their broad absorption profiles, tunable emission wavelengths and high photooxidation stability.[10-13] Herein, we modified QDs with hemin and applied them to observe the process and infer the mechanism of heme absorption *in vivo* and *in vitro*. Moreover, we demonstrated HCP1 pathway is critical and predominant in heme-iron absorption, which would provide strategies in designing targeted heme iron supplements in the future.

Methods

Preparation and characterization of QDs-hemin

Water-soluble QDs (QDs-NH_2) were prepared as follows: oil-soluble QDs (Wuhan Jiayuan Co. Ltd., Hubei, China) were washed with anhydrous alcohol and dissolved in chloroform together with PEG (2000)-NH_2 (Avanti Polar Lipids, Alabaster, USA). The mixture was sonicated for 1-2 min and chloroform was evaporated on a rotary evaporator at room temperature. Finally, Britton-Robinson buffer (pH = 8.4) was added to the free-chloroform flask to obtain QDs-NH_2. QDs-hemin were synthesized by mixing QDs-NH_2, hemin (their mole ratio 1:20) and EDC (Sigma-Aldrich St. Louis, MO, USA) at orbital shaker (TS-1, Kylin-Bell Co., Jiangsu, China) for 3 h in the dark, subsequently the solution was ultrafiltrated. To determine the joint efficiency of QDs-NH_2 to hemin, the UV–vis absorption and fluorescence spectra of oil-soluble QDs, water-soluble QDs-NH_2, QDs-hemin and hemin were acquired using a UV–vis spectrometer (U-3010, Hitachi Co., Tokyo, Japan) and fluorescence spectrophotometer (F-2500, Hitachi Co.). The UV–vis spectral data were imported into Orthogonal Projection Approach (OPA) in the MATLAB environment, and the accurate concentration of QDs-NH_2 and hemin in the QDs-hemin was calculated. The microstructure of QDs-hemin was characterized by TEM, and the diameter and zeta potential were measured using a ZetaSizer Nano series Nano-ZS (Malvern Instruments Ltd, Malvern, UK). The fluorescence stability of QDs-hemin was examined at Britton-Robinson (B. R.) buffer (pH = 5.0~9.0) and NaCl solution (0~1.0 M).

Animals and treatments

Kunming (KM) mice, weighing 25 g, were purchased from Hebei Medical University. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethical and Welfare Committee of Hebei Normal Univeristy (approval no. IACUC-137002). Duodenal loops were made as previously described.[14] Briefly, two sterile cotton threads were ligated at the duodenum of anesthetized mice (pentobarbital sodium, 40 mg/kg, i.p.). One was placed distal to the pylorus, and the other was 2.5 cm away from the first ligature. The closed loops were then filled with hemin solution (15 mg/mL) and incubated for 20 min, 40 min and 60 min before mice were culled. The incubation time of QDs-hemin and QDs-NH_2 was for 40 min.

Absorption of QDs-hemin in mice detected by Confocal Laser Scanning Microscopy (CLSM) and Micro X-ray Fluorescence (μ-XRF)

After incubation with hemin or QDs-hemin, the duodenal loops were fixed and transverse frozen sections were cut at 8-μm thickness. Some sections were used for fluorescence detection by CLSM with a Zeiss LSM510 microscope. Others were mounted onto 3-mm-thick Mylar films (polycarbonate) for analysis with synchrotron radiation μ-XRF. The μ-XRF microspectroscopy experiment was performed on a 4W1B endstation at the Beijing Synchrotron Radiation Facility, operating at 2.5 GeV with current from 150 mA to 250 mA. The incident X-ray energy was monochromatized by a W/B4C Double- Multilayer- Monochromator (DMM) at 15 keV and focused down to 50 μm in diameter by the polycapillary lens. Two-dimensional mapping was acquired in step mode, in which the sample was held on a precision motor-driven stage while scanning 100 μm stepwise. The Si (Li) solid state detector was used to detect X-ray fluorescence emission lines with a live time of 10 s. The data reduction and process were performed using the PyMCA package.[15]

Cell culture and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

Caco-2 cell line (ATCC HTB-37), human colorectal adenocarcinoma, was maintained in minimum essential medium (MEM, Gibco) supplemented with 20% FBS (Gibco), 100 U/mL