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Secretion of intestinal goblet cells: A novel excretion pathway of nanoparticles

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Abstract

Understanding the excretion pathway is one of the most important prerequisites for the safe use of nanoparticles in biomedicine. However, the excretion of nanoparticles in animals remains largely unknown, except for some particles very small in size. Here we report a novel natural pathway for nanoparticle excretion, the intestinal goblet cell (GC) secretion pathway (IGCSP). Direct live observation of the behavior of 30-200 nm activated carbon nanoparticles (ACNP) demonstrated that ACNP microinjected into the yolk sac of zebrafish can be excreted directly through intestinal tract without involving the hepato-biliary (hap-bile) system. Histopathological examination in mice after ligation of the common bile duct (CBD) demonstrated that the intravenously-injected ACNP were excreted into the gut lumen through the secretion of intestinal GCs. ACNP in various secretion phases were revealed by histopathological examination and transmission electron microscopy (TEM). IGCSP, in combination with renal and hap-bile pathways, constitutes a complete nanoparticle excretion mechanism.

From the Clinical Editor: Nanoparticle elimination pathways are in the forefront of interest in an effort to optimize and enable nanomedicine applications. This team of authors reports a novel natural pathway for nanoparticle excretion, the intestinal goblet cell (GC) secretion pathway (IGCSP). Direct live observation of the behavior of activated carbon nanoparticles has shown excretion directly through the intestinal tract without involving the hepato-biliary (hap-bile) system in a zebrafish model.

Key words: Nanoparticle; Excretion; Pathway; Goblet cell; Aggregation

The understanding of the excretion pathway becomes more and more important with the development of nanotechnology and the application of nanomaterials in biomedicine. It is important not only for the biosafety issue of engineered nanoparticles but also for the practical use of nanoparticles as diagnostic and therapeutic agents or as drug carriers, because excretion is the best way to cease the action of nanoparticles on tissues and cells. Based on the understanding of the excretion pathway, appropriate protocols can be worked out to deal with the nanoparticles once they entered the animal body. Unfortunately, such pathway remains poorly understood, though there are many literatures on the clearance of nanoparticles from blood[1,2] or tissues such as lung[3,4] and liver.[5] These experiments provide information concerning the clearance mechanism to remove particles from local tissues rather than information on systemic excretion of nanoparticles.[6] Few studies have reported two main excretion pathways of intravenously-injected nanoparticles, the kidney–urine pathway and hepatobiliary system (HBS)–feces pathway. Kidney excretion of nanoparticles is limited to very small ones, such as quantum dots[7] and fullerines.[8] HBS excretes some larger nanoparticles, but the clearance rate is no more than 1% within 24 h, and there is an inverse relation between HBS excretion and sizes.[9] However, Manabe et al found that 500 nm latex particles can be cleared from medaca embryos,[10] suggesting that there are some other excretion pathways for nanoparticle excretion without involving the HBS. Souris et al reported that the concentration of intravenously-injected 50-100 nm silica nanoparticles in liver

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was much lower than that in intestinal wall and feces.[11] This inconsistence in quantity does not support the hypothesis that HBS plays main roles in the intestinal excretion of nanoparticles. On the contrary, the high concentration of nanoparticles in intestinal wall[11] suggested that the intestinal wall may play important roles in the excretion of nanoparticles through intestinal tract. GCs are one of the four main cell types[12] in the intestinal membrane and have the ability to entrap nanoparticles.[13] Recently, there have been several studies on the interactions between GCs and orally-administered nanoparticles.[13–16] GCs can uptake nanoparticle,[17] which makes GCs the most possible candidate cells playing roles in the intestinal excretion of nanoparticles. Our earlier studies found that intravenously-injected nanoparticles had distribution in intestinal GCs[18] and hypothesized that intestinal wall may play roles in the excretion of nanoparticles but did not investigate in detail.[18,19] The present work carefully examined the mechanism for intestinal excretion of nanoparticles and revealed a novel pathway, IGCSP, for nanoparticle excretion.

Methods

Preparation and characterization of ACNP

ACNP were prepared from medicinal activated carbon (MAC; Haichangqing Co. Ltd, Beijing, China) by a top-down method (supplemental materials). The diameters of ACNP were determined by atomic force microscope (AFM), scanning electron microscope (SEM) and Laser Particle Size and Zeta Potential Analyzer. The internal crystal structures were detected by X-ray defraction (XRD). For injection, 1 mg ACNP was added into 100 mL normal saline and suspended in an ultrasound field of 40 Hz, 180 W for 20 min.

Handling of the animals

Zebrafish eggs were collected, cleaned, and washed with egg-water[20] within one hour after fertilization. Eggs from different females were pooled, placed in 7 cm sterile Petri dishes containing eggwater, and incubated at 28.0 to 28.5 °C. Embryonic fish were stocked in an Aquatic Habitat re-circulating tank system at 28.5 °C with a 14 h light/10 h dark cycle. The water was purified by reverse osmosis and adjusted to pH 7 and conductivity of 350 μS. The mice were handled as described previously.[21] Briefly, 20 Kunming mice (20-25 g in body weight) were constantly monitored and fed fluid nutritional diet free from pathogens and particulate materials for 10 days before ACNP treatment to avoid the influences of ingested particles on the observation of ACNP. All the animal procedures were approved by the Animal Subject Review Committee of the Beijing Academy of Medical Science.

Pigment inhibition in zebrafish

Pigmentation genes were suppressed by adding 1-phenyl, 2-thiourea (PTU) (Sigma, USA) into fish water in a final concentration of 75 μM in the developmental stage of 28 somites.[22] This concentration produced enough pigment inhibition with no adverse effects on the hatching and survival.

Microinjection and observation of ACNP in zebrafish

Embryonic zebrafish at the age of 24 h were put into a 7-cm culture dish and anesthetized by addition of tricaine methanesulfonate into the dish at a final concentration of 0.64 mM. The anesthetized zebrafish were placed in the slanting grooves of a silica gel sheet. Excessive water around the fish was absorbed with filter papers to leave just enough water to bathe the fish body. Five μL suspension of ACNP at the concentration of 5 mg/mL was injected into the yolk sac of zebrafish in an IM 300 microinjection instrument (Narishige, Japan) (Figure S3). All ACNP suspensions were freshly dispersed by ultrasonication for 10 min before use. During the whole period of experiment, zebrafish that received microinjection of ACNP exhibited no significant differences in death rate, development, teratogenesis, and cardiovascular toxicity in comparison with control (Figure S4).

Ligation of common bile duct and injection of ACNP into the mice

The common bile duct was ligated with the method originally described by Cameron and Oakley[23] with modifications. Briefly, Kunming mice of 20-25 g were anaesthetized with pentobarbital (25 mg/kg) and fixed onto a wood surgical sheet. A mid-abdominal incision was made, and the abdominal tissues were separated carefully to clearly expose the CBD. Two sterile nylon medical surgical sutures (Unic Surgical Sutures, Mfg., Co., Ltd., Suzhou, China), 0.2 mm in diameter, were put through under the CBD, and two nodes were made at both ends of a segment of CBD (Figure S5), and the CBD was then cut off between the two ends. After closure of abdomen, ACNP were suspended in 0.9% NaCl to a final concentration of 5 mg/mL and injected through tail veins in a dose of 50 mg/kg (i.e., 10 mL/kg). All the suspensions of ACNP were freshly dispersed by sonication for 10 min before use. After the injection of ACNP, the animals' body weight, behavior, and number of blood cells were monitored (Figure S6). At the end of the experiments, these physical parameters showed no significant differences from those of control (Figure S6).

Histopathological examination

On the 4th day after injection of ACNP, the mice were anaesthetized and their internal organs were taken out and fixed in 40% formaldehyde. 5 μM thick sections were made on a Lecia RM2135 Rotary Microtome. The sections were stained in hematoxylin and eosin or alcian blue and observed under an Olympus BH2 phase contrast microscope (Japan).

Quantitative evaluation of the efficiency of IGCSP for ACNP

The quantitative evaluation was carried out with rates occupied by ACNP-containing GCs in the total number of the GCs seen under a phase light microscope in 10 visions in 3 HE-stained histopathological sections, which were calculated by a formula: Rates (%) = Number of ACNP containing GCs/total number of seen GCs×100%. 120 Mice without CBD ligation were randomly divided into 4 groups and 30, 60, 100 and