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Pharmacokinetics study of Zr-89-labeled melanin nanoparticle in iron-overload mice

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ABSTRACT

Melanin, a natural biological pigment present in many organisms, has been found to exhibit multiple functions. An important property of melanin is its ability to chelate metal ions strongly, which might be developed as an iron chelator for iron overload therapy. Herein, we prepared the ultrasmall water-soluble melanin nanoparticle (MP) and firstly evaluate the pharmacokinetics of MP in iron-overload mice to provide scientific basis for treating iron-overload. To study the circulation time and biodistribution, MP was labeled with ^{89}Zr, a long half-life (78.4 h) positron-emitting metal which is suited for the labeling of nanoparticles and large bioactive molecule. MP was chelated with ^{89}Zr directly at pH 5, resulting in non-decay-corrected yield of 89.6% and a radiochemical purity of more than 98%. The specific activity was at least190 MBq/μmol. The ^{89}Zr-MP was stable in human plasma and PBS for at least 48 h. The half-life of ^{89}Zr-MP was about 15.70 ± 1.74 h in iron-overload mice. Biodistribution studies and MicroPET imaging showed that ^{89}Zr-MP mainly accumulated in liver and spleen, which are the target organ of iron-overload. The results indicate that the melanin nanoparticle is promising for further iron overload therapy.

1. Introduction

Melanin is an irregular functional biopolymer and ubiquitous biological pigment that can be found throughout the world's flora and fauna, such as human skin, hair, eyes, the feathers of birds, the ink sacs of cuttlefish, plants coloration of seeds, berries, flowers as well as in some fungi [1–3]. It has attracted much attention because of the involvement in various biological functions, including the protection of humans and animals from ultraviolet injury, antibiotic function, photosensitization, free radical quenching, metal ion chelation, and even involvement in nerve systems [4–6]. For its native optical properties, it has been used for photoacoustic imaging (PAI) and photothermal therapy (PTT) [7,8]. For its metal ion chelating ability, it has been conjugated with paramagnetic iron to enhance T1 MR signal [9], and several melanin-based materials have been developed as contrast-enhancing agents [10,11]. Recently, we firstly prepared the ultrasmall water-soluble

melanin nanoparticle (MP) and chelated it with Fe(III). The quantity of Fe(III) chelated to one MP is up to about 90 ions [7]. This gave us an idea to develop melanin as an iron chelator for iron overload therapy.

As we know, iron overload would result from long-term repeated blood transfusions for the treatment of hematological disease such as thalassemias, sickle cell anemia and myelodysplastic syndromes (MDS) [12,13]. Since humans lack of iron excretion pathway, excess iron is harmful to the organism as it is deposited in organs and the vasculature [14]. Hence, exogenous chelator such as desferoxamine (DFO) has been used to treat iron overload. However, the chelate ratio of iron to DFO is only 1. Moreover, the short half-life (5 min) and poor bioavailability lead to frequent doses and serious side effects [15,16].

To resolve these problems, we want to use MP instead. Pharmacokinetics is very important for new drug development. Generally, the research about the pharmacokinetics is measured by HPLC or LC–MS. However, due to the difficulty of separation from biosamples and the broad-spectrum light absorption property [2,17,18], MP could not be determined through traditional methods. Isotope radiolabeling could provide accurate pharmacokinetic profiling for macromolecular drug such as protein, liposome, polymer and so on [19,20]. Image-based quantitation of drugs generated by positron emission tomography (PET) also enables real-time non-invasive data analysis [21]. ^{89}Zr is a new positron-emitting radiometal with a long half-life of 78.4 h. It is promising for the labeling of slowly-accumulating substance, making

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it ideally suitable for in vivo imaging of antibodies, nanoparticles and other large bioactive molecule [22,23]. Moreover, because of the strong ability of melanin to chelate metal ions, the labeling procedures would be facile. Herein, MP was designed and labeled with ^{89}Zr. The stability and the pharmacokinetic profile of ^{89}Zr-MP were evaluated.

2. Materials and methods

2.1. Materials

All commercially obtained chemicals were of analytical grade and used without further purification unless specified. The following re-agents were acquired and used as received: Iron-dextran (100 mg/mL, Sigma–Aldrich), melanin (Sigma–Aldrich), methoxypolyethyleneglycol amine (PEG$_{2000}$-NH$_2$, MW = 2000, LaysanBio). Dialysis bag (MWCO: 3500, Spectrum Laboratories, USA), phosphate buffered saline (PBS, Gibco) and Millipore water (18.2 MΩ cm) was used. PD-10 column, NAP-5 columns were purchased from GE Health, and ^{89}Zr was obtained from BV Cyclotron VU (The Netherlands). ICR mice (6 ~ 8 weeks old) were purchased from SLAC Laboratory Animal Co., Ltd., China for in vivo studies. All animal studies were conducted under a protocol ap-proved by the local animal welfare committee and performed according to national regulations.

2.2. Preparation and purification of MP

The water-soluble PEG$_{2000}$-functionalized melanin nanoparticles (MP) were prepared according to the previous report [7,24,25]. In brief, commercial melanin granules (20 mg) were dissolved completely by adding 10 mL 0.1 M NaOH solution. Under strong sonication, about 7 ml 0.1 M HCl solution was quickly added to adjust pH to 7. After puri-fication and lyophilization, black solid and water-soluable melanin nanoparticle was obtained. Then, under pH 9.5, PEG$_{2000}$-NH$_2$ (50 mg) aqueous solution was dropped into the melanin particle aqueous solu-tion (1 mg/mL) and stirred for 12 h vigorously. The crude product was purified by a centrifugal-filter (Amicon centrifugal filter device, MWCO = 30 kDa). After purification, MP were freeze-dried and weighed to preliminary calculate the quantity of the PEG attached on MP. The size of MP was measured by Dynamic Light Scattering (DLS) instrument (ZetaSizer Nano-ZS, Malvern). The morphologies were ob-tained from transmission electronic microscope (TEM, JEOL JEM-2010). The molecular weight of MP was determined by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS).

2.3. Radiolabeling MP with ^{89}Zr

^{89}Zr (IV) oxalate (37 MBq, 50 μL) in 1 M oxalic acid was diluted with 200 μL PBS and the pH was adjusted to 5 by 1 M NaOH (25 μL). 300 μL MP solution (10 mg/mL) was added directly and the mixture was incu-bated at 37 °C for 30 min. After the reaction, the radiolabeled product was purified using a PD-10 desalting column (GE Healthcare) to remove free ^{89}Zr. The product was washed out by PBS and collected by eppendorf tube. The eluted fractions were measured for radioactivity and the most concentrated fractions were diluted with normal saline and then passed through a 0.22 μm Millipore filter into a sterile vial for animal experiments. According to the reported methods [26–27], the radiochemical purity was determined by instant thin layer chroma-tography (ITLC) and a Mini-TLC scanner (BIOSCAN, USA). GF-254 silica gel plates were used as the stationary phase and citrate buffer (0.1 M, pH 5) was used as the mobile phase. Under the condition ^{89}Zr-MP remained at the origin (Rf = 0) whereas free ^{89}Zr migrated to the solvent front.

2.4. In vitro stability studies

The stability of ^{89}Zr chelated MP was determined by incubating the radiolabeled compound in PBS and human plasma at 37 °C respectively. The purified ^{89}Zr-MP (200 μL/3.7 MBq/0.5 mg, respectively) was placed in a dialysis bag (MWCO 10 K) and then suspended into 20 mL plasma or PBS with magnetic stirring. At various time intervals (2, 4, 16, 24 and 48 h), 0.5 mL dialysate was taken out to calculate the radioactivity of those leaked into the plasma or PBS by PerkinElmer 1470 γ-counter, and 0.5 mL fresh plasma or PBS was added then.

2.5. Establishment of iron overload model

The model of iron overload was established according to the method of Muhammad [28]. On the first day, ICR mice (6 ~ 8 weeks old) were injected intravenously with Iron dextran (150 mg/kg of Fe, 200 μL/20 g) via the tail vein. A week waiting period was required to make the deposition of iron into the organs and excrete unabsorbed Iron dextran.

2.6. Pharmacokinetics study of ^{89}Zr-MP

Iron overload mice (6 ~ 8 weeks old, n = 6) were injected intrave-nously with ^{89}Zr-MP (100 μL/3.7 MBq/0.5 mg) to a prescribed dose of 25 mg/kg. Blood (10 μL) was collected at various time intervals after in-jection (0.25, 0.5, 1, 1.5, 2.5, 3.5, 4, 6, 15, 24 and 48 h). The concentration of ^{89}Zr-MP in blood was calculated from the radioactivity measured by the γ-counter (PerkinElmer). The pharmacokinetic parameters were analyzed with a two-compartmental open model using DAS2.0 soft-ware. The mice were sacrificed at 48 h after injection, and major organs were collected and wet-weighed. The radioactivity of the tissue was de-termined by using the γ-counter to calculate uptake as the percentage injected dose per gram of tissue (%ID/g).

2.7. PET studies of ^{89}Zr-MP

Small animal PET studies were performed with a micro-PET scanner (Inveon, Siemens) according to the previous report [25]. Iron overload mice were injected with ^{89}Zr-MP (100 μL/3.7 MBq/0.5 mg) intravenous-ly and anesthetized with isoflurane (n = 6) then. PET images were ac-quired for 10 min at different times (2, 4, 12, 24 and 48 h) after injection. The images were acquired and reconstructed by a three-dimensional ordered subset expectation maximum (OSEM) algorithm. For PET quantitative analysis, regions of interest (ROIs) over the major organs were drawn on decay-corrected whole-body coronal images. Then the %ID/g was derived from the readings using vendor software ASI Pro 6.7.1.1.

2.8. Statistical analysis

Statistical analysis was performed with Graphpad prism 6. Quantita-tive data are expressed as mean ± SD, and one-way analysis of variance was used to determine statistical differences. Paired comparisons were considered significant if p < 0.05.

3. Results and discussion

3.1. Synthesis and characterization of MP

The natural melanin has poor water-solubility, and Fe^{3+} is a strong cross-linker for catechol group in melanin that would result in the for-mation of MP precipitation [29]. Therefore, the pristine melanin granule was first dissolved in a 0.1 N NaOH for preparing the plain water-soluble MP, and then modified by PEG-NH2 to retain the water-solubility and avoid the formation of metal ion-initiated precipitation efficiently [30]. The quantity of the PEG attached on MP is about 27 and the average