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Fe–salphen complexes from intracellular pH-triggered degradation of Fe_3O_4@Salphen-In^{III} CPPs for selectively killing cancer cells

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ABSTRACT

We propose a modular synthetic strategy to constitute metallosalphen prodrugs in the form of coordination polymer nanoparticles, comprising magnetite nanocrystal colloidal cluster as core and salphen-In^{III} coordination polymer as shell. These composite nanoparticles are not only equipped with intense photoluminescence, sensitive magnetic responsiveness and pH-dependent degradability, but also serve as prodrugs to accomplish intercellular conversion from non-toxic nanoparticles (Fe_3O_4@Salphen-In^{III}) to pharmacologically active complexes (Fe–salphen), allowing to specifically inhibit the proliferation of A549 cancer cells via caspase activation.

1. Introduction

Medical inorganic chemistry is a thriving area of research [1–7], which was initially fueled by the serendipitous discovery of the cisplatin anti-proliferative activity [8].Tremendous efforts have been dedicated to exploring a variety of metal complexes in pharmaceutical use [1–11]. From the point of view of synthesis, metal complexes offer a versatile platform for design of anticancer agents due to the distinct characteristic of central metal ions, such as multiple coordination numbers, accessible redox states, flexible ligand substitutions, and diverse geometries. Of these members, cisplatin is one of the leading metal-based chemotherapeutics, being pronouncedly potent against a wide spectrum of cancer cell lines while used alone or in combination with other drugs [12]. Significant toxicity side effects and drug resistances, however, have limited its clinical applications [13,14]. There is a need, thereby, for new metallodrugs that are aimed at the lower side effects as well as specificity and efficacy in cancer therapies. Linppard et al. prepared a polymeric nanoparticle to encapsulate the water-soluble Pt(IV) prodrug by double emulsion for the purpose of reduced toxic side effect and controlled release [15]. Metallo-salen/salphen complexes are widely used as catalysts in selective oxidation, organic epoxidation, CO_2 fixation, and so on [16]. More intriguingly, their notable apoptotic and antitumor activities have been specifically investigated in the primary studies, which elucidated that functions of central metal ions (e.g. Mn^{III}, Fe^{II}, and Fe^{III}) and substituents of salen/salphen ligands were both responsible for tumor-selective apoptosis and cytotoxicity toward cisplatin-resistant cancer cells [17–21]. Albeit with the achievements of metallo-salen/salphen complexes, there still exist some major drawbacks, i.e., large dose of administration, poor water solubility, short circulating time and low bioavailability, all of which would elicit pharmacological deficiencies and deleterious effects [22]. To circumvent these issues, rationally designed drug delivery systems rather than new metal complexes should be greatly developed with the aim of enhancing the performance profile of current metallodrugs. Meanwhile, “safe” delivery of metal complexes to their targets also poses one crucial challenge in cancer chemotherapy, owing to their strong coordination interaction with specific biomolecules [23].

Since Mirkin et al. pioneered the study in synthesis of coordination polymer particles (CPPs), CPPs arouse mounting interests in a wide range of fields [24,25]; they promise great potentials for gas

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adsorption [26–28], drug delivery [29–34], biological detection [35], optical imaging [36–40], supercapacitive storage [41] and heterogeneous catalysis [42,43]. In light of flexibility and manipulability over coordination polymerization, organic anticancer drugs could either be *in situ* encapsulated within CPPs as secondary ligands [30,33], or yield nanoparticles self-supported via the interaction of metal-ion-drug coordination [29,32,34]. Thus the innovative model of nanocarriers for drug delivery is constructed in the form of CPPs, and, essentially, acid cleavability of coordination bonds allows for the sustained, pH-responsive drug release. For the aforementioned metallodrugs, however, integration of them into CPPs has been rarely concerned thus so far. Lin et al. realized the first metallodrug-incorporated CPPs by catenating Ir ions with the decorated cisplatin, whereas the activity of chemically modified metallodrugs is compromised to some extent [31]. Therefore, we considered not to comply with the conventional formulation that usually engineers "small" metal complexes into "large" CPPs, but to envision an alternative solution to originate a modular synthesis of CPPs using constituent components of metallodrugs. Hence, we designed a well-defined core–shell Fe_3O_4@Salphen-In^{III} CPP comprising Fe_3O_4 colloidal nanocrystal cluster as core and salphen-In^{III} coordination polymer as shell with the aim of tackling systematic toxicity and side effects as well as enhancing site-specific delivery of metallodrugs.

2. Materials and methods

2.1. Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate (NaOAc), ethylene glycol (EG), trisodium citrate dihydrate ($Na_3Cit \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagents Co. Ltd. Indium nitrate hydrate ($In(NO_3)_3 \cdot xH_2O$) was purchased from Aladdin Reagent Company. Trifluoroacetic acid (99.8%) was purchased from Merck (Darmstadt, Germany). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Japan). Lung carcinoma cell (A549) was obtained from the American Type Culture Collection (ATCC). All the reagents were analytical grade and used as received. Deionized water (Millipore) of resistivity greater than 18.0 MΩ cm were used all through the experiments. The modified salphen ligands, *N,N′*-phenylenebis(salicylideneimine) dicarboxylic acid, were synthesized according to the literature [44]. Salphen-In^{III} CPPs were prepared by following the known method [45].

2.2. Synthesis of citrate-stabilized MCNCs

MCNCs were prepared through a solvothermal process [46]. Typically, 2.2 g $FeCl_3 \cdot 6H_2O$, 2.6 g NaOAc, and 0.48 g Na_3Cit were dissolved in 40 mL of EG to form a homogeneous yellow dispersion with the aid of ultrasonication. The mixture was then stirred vigorously at 160 °C for 1 h. After that, it was instantly transferred into a Teflon-lined stainless-steel autoclave with 50 mL capacity. The reaction was allowed to proceed at 200 °C in an oven for 15 h. After the solvothermal process, the precipitates were collected by a magnet and rinsed several times with ethanol and deionized water to remove residues. The products were dried under vacuum for further use.

2.3. Synthesis of Fe_3O_4@Salphen-In^{III} CPPs

The carboxylate-modified salphen ligands (6 mg, 15 mmol) and $In(NO_3)_3 \cdot xH_2O$ (4.47 mg, 15 mmol) were dissolved in 4 mL DMF to form a yellow solution, to which 8 mg MCNC powder was added. The above precursor mixture was ultrasonicated, transferred to a 25 mL three-necked flask, and stirred vigorously at 120 °C. The reaction was allowed to proceed for 15 min, during which the brownish precipitates were gradually formed in the mixture. After cooling to ambient temperature, the products were collected by an applied magnet and washed with DMF and deionized water, respectively.

2.4. Characterization

High resolution transmission electron microscopy (HR TEM) images were taken on a JEM-2010 (JEOL, Japan) transmission electron microscope at an accelerating voltage of 200 kV. Samples dispersed at an appropriate concentration were cast onto a carbon-coated copper grid. Magnetic characterization was carried out with a vibrating sample magnetometer on a Model 6000 physical property measurement system (Quantum Design, USA) at 300 K. Fourier transform infrared (FT IR) spectra were recorder on a Magna-550 (Nicolet) spectrometer. The dried samples were mixed with KBr, and they were compressed to a plate for measurement. TG analysis was obtained with a Pyrisis-1 (Perkin–Elmer, USA) thermal analysis system under a flowing air atmosphere at a heating rate of 20 °C min^{-1} from 100 to 800 °C. Fluorescence spectra were obtained at room temperature using an FLS920 spectrofluorimeter. MALDI-TOF mass spectrometry analysis was performed in positive reflection mode on a 5800 Proteomic Analyzer (Applied Biosystems, Framingham, MA, USA) with a Nd:YAG laser at 355 nm, a repetition rate of 200 Hz, and an acceleration voltage of 20 kV. Nitrogen sorption isotherms were obtained on an ASAP2020 (Micromeritics, USA) accelerated surface area analyzer at 77 K. Before measurements, the samples were degassed under vacuum at 200 °C for at least 6 h. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. By using the Barrett-Joyner-Halenda (BJH) model, the pore size distributions were derived from the desorption branches of isotherms, and the total pore volumes were estimated from the adsorbed volume at a relative pressure of 0.971.

2.5. Cell viability assay

All cells were cultured in complete 1640 medium, with 10% (v/v) fetal bovine serum (FBS), at 37 °C, 5% CO_2 and 10% humidity. Cell viability was measured using a Cell Counting Kit-8. Firstly, cells were seeded into 96-well plates (Costar, Corning, NY). After incubating (37 °C, 5% CO_2 and 10% humidity) for 24 h, the culture medium was removed and replaced with the complete medium containing 0, 1, 5, 10, 20 and 50 μM salphen-In^{III} CPPs, Fe_3O_4 and Fe_3O_4@Salphen-In^{III} CPPs, respectively. Moreover, cell viability was assessed after incubation times of 12, 24, and 48 h. Meanwhile, the wells unexposed to any samples were regarded as control. Then a mixture of the tetrazolium reagent (from the Cell Counting Kit-8) and the complete medium (1:10) was added into each well. Finally, cell viability was calculated as the absorbance ratio between test and control wells. The absorbance at 450 nm was measured and referenced with that at 650 nm by Infinite M200 microplate reader (Tecan, Durham, USA).

2.6. Study of the change in cell morphology

Firstly, cells were seeded into 6-well plates. After 24-h incubating (37 °C, 5% CO_2 and 10% humidity), the culture medium was replaced with complete medium containing 50 μM Fe_3O_4@Salphen-In^{III} CPPs. After another 24 h, adherent cells were washed with PBS for three times. The nucleus was labeled with 4 μg mL^{-1} Hoechst 33,342 for 5 min, and then the cells were washed and observed using fluorescence microscope at ×10 magnification.

2.7. Quantitative measurement for Fe_3O_4@Salphen-In^{III} CPPs in cellular uptake

A549 and 16HBE cells were cultured with 2 mL of complete medium in 6-well plates at a density of 2×10^5 cells mL^{-1}. After cultured for 24 h at 37 °C, 5% CO_2 and 10% humidity, the medium was removed and changed to the complete medium, respectively, containing 10 μM and 20 μM Fe_3O_4@Salphen-In^{III} CPPs, for internalization experiment. The cells were incubated for different time slice including 1, 3, 6, 12, 24 and 48 h, four wells of cells at each time point. Cells in each well were gently washed three times with PBS, digested with 0.25% trypsin containing 0.02% EDTA, centrifuged for 10 min at 1500 rpm, collected and counted. Afterwards, 3 mL HNO_3 was added to each cell sample, and then transferred to flasks for pre-digestion overnight. In the next day, 2 mL 30% H_2O_2 was added to each flask. The flasks were placed onto a hot plate and maintained at 150 °C for 3 h until digestion was complete, and then they were cooled to room temperature. The solution in each flask was diluted to 5 mL with 2% HNO_3. A series of Fe standard solutions (0, 0.1, 0.5, 1, 5, 10, 50 and 100 ppm) were prepared with the above solution. Both standard and test solutions were measured by inductively coupled plasma mass spectrometry (ICP-MS, Thermal Elemental X7, Thermal Fisher Scientific Inc, USA).

2.8. Study of pH changes in lysosome

Cells were seeded into 6-well plates for 24 h at 37 °C, 5% CO_2 and 10% humidity. After that, cells were incubated with 2 μM LysoSensor™ Green DND-189 for 40 min prior to rinsing three times with PBS. Then pH of lysosome was analyzed when 20 μM Fe_3O_4@Salphen-In^{III} CPPs were incubated in complete medium for different time slice (1, 2, 3, 4, 5, 6, 8, 10 and 12 h). Finally, cells were digested, collected, acquired on flow cytometry (BD FACS Calibur) and analyzed with FCS Express software.

2.9. Assessment of the integrity of lysosomal membrane

Cells were seeded into 35 mm petri-dishes for 24 h at 37 °C, 5% CO_2 and 10% humidity. Cells were then incubated for 15 min in the complete medium containing 5 μg mL^{-1} AO and 10% FBS before rinsed three times with PBS. Lysosomal membrane permeation of the two cells was analyzed after 24-h treatment with a complete medium containing 50 μM Fe_3O_4@Salphen-In^{III} CPPs and 2 μg mL^{-1} PEI (as positive control), respectively. Finally, the dead cells were removed by wash and the remaining cells were observed using confocal microscope (Perkin Elmer Ultra View Vox system, USA). The emission of samples was detected at 537 nm (green) and 615 nm (red) with excitation wavelength of 488 nm. Identically, the cells were stained with AO, digested, collected and measured by flow cytometry using an excitation wavelength of 488 nm and an emission wavelength of 670 nm.