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Indole conjugated silica and magnetic nanoparticles as inhibitors of HIF

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ABSTRACT

Multifunctional silica nano-vehicles (SiO_2@indol-IL) and magnetic nanoparticles (Fe_3O_4@indol-IL) were constructed through the Schiff bases condensation of indole-3-carboxaldehyde and 4-acetyl-N-allyl pyridinium chloride (ILs) with the amine groups of silica and magnetic nanoparticles. SiO_2@indol-IL can inhibit the proliferation of HepG-2 cells in 48 h. Fe_3O_4@indol-IL can mimic the function of catalase to disproportionate H_2O_2 to O_2, and has obvious effect on the proliferation of HepG-2 cells in 72 h. Moreover, the two nanoparticles show some inhibition on the expression of hypoxia inducible factor (HIF-1α), glucose transporter (GLUT1) and the production of lactate in HepG-2 cells. Therefore, we deduced that indole conjugated silica and magnetic nanparticles could be used as inhibitors of HIF-1α or GLUT1.

1. Introduction

Cancer cells typically display altered glucose metabolism characterized by a preference of aerobic glycolysis, known as the Warburg effect, which facilitates cell proliferation [1]. Hypoxia inducible factors (HIFs) are heterodimeric transcription factors induced in many cancers where they frequently promote the expression of protumourigenic pathways [2]. Under hypoxia, mitochondrial function alterations such as decreased production of ATP, calcium buffering capacity, stimulation of reactive oxygen species, loss of membrane potential, and release of pro-apoptotic proteins from the mitochondrial intermembrane space, are involved while glycolysis will become enhanced [3,4]. Glycolysis allows for continued ATP production without O_2-dependent oxidative phosphorylation, and is thus an important pathway under hypoxic conditions [5]. The glycolytic inhibitors are based predominantly on ATP depletion and particularly effective against cancer cells with mitochondrial defects under hypoxic conditions [6,7]. A successful compound that has been shown to block aerobic glycolysis in cancer cells, possibly by inhibiting hexokinase, is lonidamine, a derivative from indazole-3-carboxylic acid. Lonidamine disrupts energy metabolism in cells resulting in ATP depletion, and in addition, leads to an accumulation of lactate in the cells [7]. In normal tissues, lactate generation is limited to anaerobic conditions where oxygen levels are low. In contrast, cancer cells preferentially convert glucose into lactate through glycolysis, even under normal oxygen concentrations, a phenomenon termed "aerobic glycolysis" or the Warburg effect [8,9].

The indole nucleus is a common substructure of many biologically active compounds and occupies an important position in medicinally relevant heterocyclic systems. Schiff bases with the structure of —C═N-(azomethine group) obtained through the condensation of amines and amino acids with 1H-indole-3-carboxaldehyde have received considerable attention since the discovery of their cytotoxic activity against cancer cell and bacteriostatic effects [10]. They show biological applications including antibacterial and antitumour activity [11,12]. However, these compounds have some effect on normal cells. Since traditional anticancer compounds cannot discern between cancer and healthy cells, the drug dose within cancerous cells is always limited, and systemic toxicity with undesired side effects is inevitable. In order to improve the therapeutic index of drugs in cancer therapy, enormous research efforts have been made to develop ideal drug carriers for tumour-targeted drug delivery [13–15]. Mesoporous magnetic nanoparticles (Fe_3O_4) and hollow mesoporous silica spheres (HMS) have been used as drug carriers because of their low toxicity. Ionic liquids (ILs) modified nano hollow silica nanoparticles were synthesized and used as host to construct

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nano-mimics of catalase [16]. Based on the mentioned properties of Schiff bases, indole analogues and nanoparticles, indole conjugated nanosilica and magnetic Fe_3O_4 nanoparticles were constructed. We report herein their synthesis, spectroscopic characterization as well as their effect on the expression of HIF.

2. Experimental

2.1. Materials

Indole-3-carboxaldehyde was purchased from Sigma. 4-Acetyl-N-allyl pyridinium chloride (IL) and amine functionalized silica SiO_2@NH_2 nanoparticles were synthesized as reported [16] and solvents were of analytical grade. Water was purified with a Millipore Milli-Q system (25 °C: 18.2 MΩ cm, 7.20×10^2 N/m). Mesoporous magnetic particles Fe_3O_4@NH_2 were synthesized using hydrothermo methods as reported [17].

2.2. Nanoparticle physical characterization

FT-IR characterizations were performed using a Nicolet Nexus 470 FT-IR spectrophotometer in the wavenumber range of 4000–400 cm^{-1}. The surface charge of the nanoparticles was investigated through zeta potentials measurements (Malvern Instruments, Zetasizer Nano ZS90). The electronic absorption spectrum was recorded using a UV-2450 UV-vis spectrophotometer at room temperature. TEM was performed at room temperature on a JEOL JEM-200CX transmission electron microscope using an accelerating voltage of 200 kV. Mean particle diameter and size distribution of the nanoparticles were measured by dynamic light scattering (DLS) using a Brookhaven 90 plus particle size analyzer.

2.3. Synthesis of SiO_2@indol-IL

The silica nanoparticles SiO_2@NH_2 were functionalized with indole-3-carboxaldehyde and ILs through the formation of Schiff base. The SiO_2@NH_2 nanoparticle (0.10 g) was dispersed in ethanol (10 mL) and mixed with 39.7 mg of indole-3-carboxaldehyde. After the mixture was heated for 8 h at 80 °C, the mixture was cooled to room temperature, and then SiO_2@indole nanoparticles were obtained by centrifugation and washed with ethanol to remove the free indole-3-carboxaldehyde. Next, 4-acetyl-N-allyl pyridinium chloride (ILs) (0.068 g, 0.014 mmol) was added into a stirred ethanol solution (10 mL) of SiO_2@indole nanoparticles (100 mg), and then the mixture was allowed to reflux for 6 h at 70 °C. The resulting precipitate (SiO_2@indol-IL) was collected by centrifugation, washed with water to remove the unreacted IL, and dried under vacuum. The amount of modified indole-3-carboxaldehyde (or 4-acetyl-N-propenylpyridinium chloride) was evaluated by measuring the absorbance of SiO_2@indol-IL at 292 nm (or 265 nm) using free indole-3-carboxaldehyde (or 4-acetyl-N-propenylpyridinium chloride) as control. The amount of loaded indole-3-carboxaldehyde and 4-acetyl-N-propenylpyridinium chloride is about 26.6% and 11.1%, respectively.

2.4. Synthesis of Fe_3O_4@indol-IL

Mesoporous magnetic particles Fe_3O_4@NH_2 were functionalized with indole-3-carboxaldehyde and ILs through the formation of Schiff base. The Fe_3O_4@NH_2 nanoparticle (0.20 g) was dispersed in ethanol (10 mL) and mixed with 48.7 mg of indole-3-carboxaldehyde for 8 h at 60 °C. After the mixture was cooled to room temperature, Fe_3O_4@indole nanoparticles were obtained by centrifugation and washed with ethanol to remove the free indole-3-carboxaldehyde. Next, 4-acetyl-N-propenylpyridinium chloride (ILs) (0.068 g, 0.014 mmol) was added into a stirred ethanol solution (10 mL) of Fe_3O_4@indole nanoparticles (100 mg), and then the mixture was allowed to reflux for 6 h at 70 °C. The resulting precipitate (Fe_3O_4@indol-IL) was collected by centrifugation, washed with water to remove the unreacted IL, and dried under vacuum. The amount of modified indole-3-carboxaldehyde (or 4-acetyl-N-propenylpyridinium chloride) was evaluated by measuring the absorbance of Fe_3O_4@indol-IL at 292 nm (or 265 nm) using free indole-3-carboxaldehyde (or 4-acetyl-N-propenylpyridinium chloride) as control. The amount of loaded indole-3-carboxaldehyde and 4-acetyl-N-propenylpyridinium chloride is about 16.4% and 10.2%, respectively.

2.5. Catalase-like activity

All of the reactions between the nanoparticles and dihydrogen peroxide were performed in buffered (Tris/Tris–HCl, 0.1 mol L^{-1}, $NaClO_4$ 0.1 mol L^{-1}, pH = 7.1) solutions at 37 °C. The volumetric measurements of the evolved dioxygen produced during the reaction of the nanoparticles with H_2O_2 were performed in triplicate as follows: a 10 mL round-bottom flask containing nanoparticles with Fe(III) ions (5×10^{-4} mol L^{-1}, 3.0 mL) in a buffered system was placed in a water (310.0 ± 0.1 K) bath. The flask was closed with a rubber septum, and a cannula was used to connect the reaction flask to an inverted graduated pipette, filled with water. While the solution containing the nanoparticles was stirred, a solution of 0.5 mL of H_2O_2 was added through the septum using a microsyringe. The volume of oxygen produced was measured in the pipette. The kinetic measurement for nanoparticles was performed in Tris/Tris–HCl solution at 37 °C. Different concentrations of dihydrogen peroxide were prepared by diluting the 30% H_2O_2 aqueous solution with Tris/Tris–HCl solution. The optimum reaction order of the substrate H_2O_2 with respect to the nanoparticles was determined by different concentrations of nanoparticles with a constant concentration of substrate H_2O_2. Similarly, the optimum reaction order of the nanoparticles with respect to the substrate H_2O_2 was determined by different concentrations of substrate H_2O_2 with a constant concentration of the nanoparticles.

2.6. Cell viability assay

The cytotoxicity assays were measured with HepG-2 cells in normal culture conditions. HepG-2 cells were seeded at a density of 4×10^4 cells/mL into sterile 96-well plates and the plates were incubated in 10% FBS-supplemented medium for 24 h. Nanoparticles (10 mg/mL) were dispersed in DMSO and diluted with culture media. After 24 h, the nanoparticles (5–60 μg/mL) were added into the cultured HepG-2 cells. Drug-containing medium was replaced with medium containing MTT (0.5 mg/mL), followed by incubation at 37 °C for 24 h, 48 h or 72 h. After removal of medium, the reduced MTT dye in each well was solubilized in 100 μL of dimethyl sulfoxide (DMSO). Cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenpyltetra-zolium bromide (MTT) assay measuring the absorbance at 570 nm. Each test was performed in triplicate.

2.7. Western blot analysis on expression level of HIF-1α in HepG-2 cells

Nuclear protein extracts were prepared from 100 mm dishes, except for experiments performed on 60 mm gas permeable dishes (Greiner Bio One, Frickenhausen, Germany), protein was isolated to perform Western analysis on Mini-Proten Tetra System (Molecular