Colloids and Surfaces B: Biointerfaces 117 (2014) 98–106

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Methotrexate intercalated layered double hydroxides with different particle sizes: Structural study and Controlled release properties

Xiao-Qing Zhang, Mei-Gui Zeng, Shu-Ping Li*, Xiao-Dong Li

Jiangsu Key Laboratory of Biofunctional Material, College of Chemistry and Material Science, Nanjing Normal University, Nanjing, 210023, China

ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form 8 February 2014
Accepted 8 February 2014
Available online 18 February 2014

Keywords:
Methotrexate
Layered double hydroxides
Nanohybrids
Controllable synthesis
Release properties

ABSTRACT

To study the influence of particle size on release properties, drug efficacy and other properties, a series of methotrexate intercalated layered double hydroxides (MTX/LDHs) nanohybrids with different particle sizes were synthesized through traditional coprecipitation method, by using the mixture of water and polyethylene glycol (volume ratio is 3:1) as solvent. The relationship between particle size and hydrothermal treatment conditions (i.e., time and temperature) had been systematically investigated, and the results indicate that the particle size can be precisely controlled between 70 and 300 nm. Elemental C/H/N and inductive coupled plasma (ICP) analysis indicated that different hydrothermal treatment almost has no effect on compositions of the nanohybrids. X-ray diffraction (XRD) patterns and fourier transform infrared spectroscopy (FTIR) investigations manifested the successful intercalation of MTX anions. MTX/LDHs particles exhibited hexagonal platelet morphology with round corner, due to the adsorption of MTX anions on positively charged LDHs surface. In addition, the crystallinity of MTX/LDHs increased with the particle diameters and the thermal stability of MTX anions was enhanced by holding together with LDHs layers. The in vitro release showed that bigger particles have much longer release duration, and the bioassay tests indicated that bigger particles are more efficient in the suppression of the tumor cells.

1. Introduction

Layered double hydroxides (LDHs) are a class of anionic clays which can be represented by the general formula of $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}A^{n-}_{x/n}\cdot mH_2O$, where M^{3+} and M^{2+} are tri- and divalent metal ions, respectively, and A^{n-} is the exchangeable anions located in the interlayers [1,2]. LDHs are the only known inorganic materials with positive layer charge, their structural units are made from stacks of positively charged octahedral sheets $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}$ and the exchangeable interlayer anions (A^{n-}) as well as water molecules. The net positive charge, which is due to substitution of M^{2+} with M^{3+} in the brucite-like metal hydroxide $M(OH)_2$, is balanced by the negative charge from the interlayer anions (A^{n-}). And then various amounts of water (mH_2O) are hydrogen bonded to the hydroxide layers or to the interlayer anions, thus forming the 3-D layered structure [3].

Indeed, thanks to LDHs intrinsic properties such as adsorption behavior, anionic exchange capacities and good biocompatibility, many processes starting from pristine LDHs precursors have already been described to access to enzyme-LDHs biohybrids [4,5]. Also LDHs have been widely used as drug and biomaterial delivery systems [6,7], e.g., many anti-inflammatory drugs such as fenbufen, diclofenac, ibuprofen and camptothecin *etc.*, were intercalated into the LDHs interlayers to form drug/LDHs nanohybrids [8,9]. In fact, drug/LDHs nanohybrids can undergo the following process very slowly under physiological conditions (pH 7.4 or smaller) to release drug, i.e.:

$$\text{drug-Mg-Al-LDHs} + H^+ \rightarrow Mg^{2+} + Al^{3+} + \text{drug} + H_2O$$

This reaction, on one hand, can release the drug slowly and then to prolong the drug effect, on the other hand, it can buffer pH falling in case of weak acidic situation, such as in endosome and lysosome after drug/LDHs are taken up by cells [10].

Here, we chose MTX as the guest drug. Methotrexate (MTX) is one of the antifolate drugs, which can effectively deactivate the metabolism of diseased cells through programmed cell death or apoptosis, and it has been applied to certain human cancers such as osteosarcoma (bone cancer) and leukemia *etc.* [11]. Unfortunately, the very short plasma half-life and high efflux rate of MTX compared to influx rate have required high administration dose, which render the clinical application restricted. Recently, lots of

* Corresponding author. Tel.: +86 25 83598280; fax: +86 25 83598678.
E-mail address: lishuping@njnu.edu.cn (S.-P. Li).

http://dx.doi.org/10.1016/j.colsurfb.2014.02.018
0927-7765/

improvement has been tried on this drug, and the synthesis of MTX intercalated LDHs nanaohybrids is proved to be the most well-established ways. Oh et al. [11,12] reported that MTX/LDHs showed better drug efficacy than MTX itself in terms of drug concentration, and the nanohybrids have higher efficiency in tumor suppression than MTX. The conventional way to get drug/LDHs nanohybrids is coprecipitation method at varied or constant pH value, followed by aging at a certain temperature [13–15], but the as-prepared nanohybrids are usually loose powders of irregular aggregates which impede the further application. On the other hand, particle size control is another important issue when LDHs materials are used in application, e.g., it seems that LDHs particles with a size between 100 and 200 nm which are individually separated in a stable suspension are desirable for gene and biomaterial carriers. Until now, little attention has been devoted to the research on how to effectively control the particle size of drug/LDHs nanohybrids and how to improve the morphology of the hybrids [16]. Another important theme which we are interested in but nobody has involved is the influence of the particle size on the release properties and the suppression effect of the tumor cells [17].

In this paper, we want to address these issues efficiently. In order to control the particle size of the nanohybrids, different hydrothermal treatment conditions (time and temperature) were performed. In order to improve the morphologies of the nanohybrids, the mixture of water and polyethylene glycol (volume ratio = 3:1) was used as solvent. Polyethylene glycol (PEG) is one of the most widely used biocompatible polymers, capable of modifying the surface of nanoparticles [18]. At last, the correlation between the properties and the particle diameters as well as the drug release mechanism has been searched for, to help us develop a new dosage form in the future.

2. Experimental

2.1. Experimental materials

Polyethylene glycol-400 (PEG-400) was purchased from Shanghai Lingfeng Chemical Reagent Co. and MTX was from Zhejiang province Huzhou prospect pharmaceutical Co. All chemicals used were of analytical grade or of the highest purity available.

2.2. Synthesis of pristine LDHs and MTX/LDHs nanohybrids with different sizes

Pristine Mg-Al-NO_3-LDHs were prepared by the typical coprecipitation method and they were used as reference materials [19]. The MTX/LDHs nanohybrids with different particle sizes were prepared in the mixture of water and PEG-400 by changing the hydrothermal treatment time and temperatures, the process was as follows: the mixed salt solution, containing 0.032 mol/L Mg^{2+} and 0.016 mol/L Al^{3+} with PEG-400/water (a volume ratio of 1:3) as solvent, was first prepared. MTX was dissolved into 15 mL 10% $NH_3{\cdot}H_2O$ to get a 0.05 mol/L solution. Then the mixed salt solution was dropped into MTX solution at a constant rate of 3 mL/min, and the final solution was adjusted to pH 9.5 by adding a certain amount of 10% $NH_3{\cdot}H_2O$. Followed by vigorously stirring for 1 h at 60 °C, the products were washed with deionized water and ethanol for several times, while N_2 gas was bubbled into the solutions throughout the coprecipitation operation. At last, they were transferred into a Telfon-lined stainless steel autoclave with variable hydrothermal treatment temperatures and time as follows: (a) 80 °C, 12 h (b) 80 °C, 24 h (c) 80 °C, 36 h (d) 80 °C, 48 h (e) 100 °C, 12 h (f) 100 °C, 24 h (g) 100 °C, 36 h and (h) 100 °C, 48 h, respectively.

2.3. Drug-loading capacity

To determine the amount of MTX loaded into LDHs, 0.01 g MTX/LDHs were dissolved by HCl solution (pH 1.2) completely and diluted to 500 mL in volumetric flask. Under this circumstance, it can be assumed that 100% of MTX is released from MTX/LDHs nanohybrid. Then the concentration of MTX was determined by monitoring the absorbance at λ_{max} = 306 nm with UV–vis spectroscopy, it must be mentioned that the concentration was calculated by regression analysis according to the standard curve obtained from a series of standard solution of MTX in HCl solution. At last, the intercalated amount of MTX was calculated, and designed as A_{In}. These data were collected in triplicate, and presented in Tables 1 and 2.

2.4. In vitro drug release

To measure the amount of MTX released from MTX/LDHs nanohybrids, the in vitro drug release test was performed as follow: 0.02 g MTX/LDHs were added into 500 mL phosphate buffer solution (pH 7.4) in a closed glass bottle at a constant temperature of 37 °C. At selected time after addition of the nanohybrids, 4 mL solution was withdrawn and centrifuged, part of the supernatant was used for the measurement, and then the concentration of MTX was measured by UV–vis spectroscopy at λ_{max} = 306 nm, at last the release profiles were plotted as the relative release percentages of MTX against time. Dissolution medium was maintained at constant volume by replacing the samples with a fresh dissolution medium [20]. These data were collected in triplicate and presented in Fig. 5A.

2.5. In vitro bioassay

Human lung adenocarcinoma cells (A549) purchased from Chinese Academy of Sciences (Shanghai, CN) were used in this study. Cells were routinely cultured at 37 °C in a humidified atmosphere with 5% CO_2 in 75 cm^2 flasks containing 10 mL of Dubecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 100 U/mL penicillin and 100 mg/mL streptomycin. When the cells were grown up to 80–90% of cellular confluence, the fault culture cells were differentiated with trypsin-EDTA and then washed twice with PBS (pH 7.4) which was prior prepared. Then the cells were diluted with a volume of DMEM containing 10% FBS. For cell proliferation and viability study, cells were seeded onto 96-well plates. Then the cells were incubated overnight at 37 °C under a 5% CO_2 atmosphere. After that the medium in the wells was replaced with fresh medium containing MTX/LDHs nanohybrids, and further incubated for 24 h. The effect of MTX/LDHs nanohybrids on cell proliferation was determined using MTT (a yellow tetrazole) assay [21]. Briefly, after the supernatant was removed, 10 μL of MTT (5 mg/mL in PBS, pH 7.4) stock solution and 90 μL DMEM with no FBS were added into each well and further incubated for 4 h at 37 °C. During the incubation, MTT was reduced to insoluble purple formazan by mitochondrial reductase in living cells. Afterwards, the product was dissolved with 100 μL of dimethylsulfoxide (DMSO). Absorbance was recorded at 570 nm on a microplate reader (Thermo MK3, USA). The MTT assays were also performed with the cells being cultured with different MTX/LDHs concentrations, and the metabolic activities of A549 cells cultured with MTX/LDHs samples of different particle sizes were also obtained. The data were collected in triplicate and presented in Fig. 6.

2.6. Characterization

XRD patterns were obtained with a D/max-2500PC rotating anode X-ray powder diffractometer (Rigaku Co. Japan), using Cu Kα radiation (λ = 1.5406 Å). The samples were scanned from 2° to