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Efficient VEGF targeting delivery of DOX using Bevacizumab conjugated SiO_2@LDH for anti-neuroblastoma therapy

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ABSTRACT

Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis and is highly expressed in carcinoma, which make it an important target for tumor targeting therapy. Neuroblastoma is the main cause for cancer-related death in children. Like most solid tumors, it is also accompanied with the overexpression of VEGF. Doxorubicin Hydrochloride (DOX), a typical chemotherapeutic agent, exhibits efficient anticancer activities for various cancers. However, DOX, without targeting ability, usually causes severe damage to normal tissues. To overcome the shortages, we designed a novel nano-composite, which is Bevacizumab (Bev) modified SiO_2@LDH nanoparticles (SiO_2@LDH-Bev), loading with DOX to achieve targeting ability and curative efficiency. SiO_2@LDH-DOX and SiO_2@LDH-Bev-DOX nanoparticles were synthesized and the physicochemical properties were characterized by TEM detection, Zeta potential analysis, FTIR, Raman and XPS analysis. Then in vitro and in vivo anti-neuroblastoma efficiency, targeting ability and mechanisms of anti-carcinoma and anti-angiogenesis of SiO_2@LDH-Bev-DOX were explored. Our results indicated that we obtained the core-shell structure SiO_2@LDH-Bev with an average diameter of 253 ± 10 nm and the amount of conjugated Bev was 4.59 ± 0.38 μg/mg SiO_2@LDH-Bev. SiO_2@LDH-Bev-DOX could improve the cellular uptake and the targeting effect of DOX to brain and tumor, enhance the anti-neuroblastoma and anti-angiogenesis efficiency both in vitro and in vivo, and alleviate side effects of DOX sharply, especially hepatic injury. In addition, we also demonstrated that angiogenesis inhibitory effect was mediated by DOX and VEGF triggered signal pathways, including PI3K/Akt, Raf/MEK/ERK, and adhesion related pathways. In summary, SiO_2@LDH-Bev could be a potential VEGF targeting nanocarrier applied in VEGF positive cancer therapy.

Statement of Significance

This paper explored that a novel core-shell structure nanomaterial SiO_2@LDH and modified SiO_2@LDH with Bevacizumab (Bev) to form a new tumor vasculature targeting nanocarrier SiO_2@LDH-Bev as vector of DOX, which was not reported before. The results indicated that SiO_2@LDH-Bev could improve the VEGF targeting ability, anti-neuroblastoma and anti-angiogenesis efficiency of DOX. At the same time, SiO_2@LDH-Bev-DOX could erase the cardiac toxicity and hepatic injury coming from DOX. Tube formation showed SiO_2@LDH-Bev-DOX had the strongest effect on inhibiting angiogenesis among all the four formulations. SiO_2@LDH-Bev-DOX could downregulate expression of p-VEGFR and inhibit activation of the Raf/MEK/ERK, p38MAPK, PI3K/Akt and FAK signaling pathways to achieve the goal of anti-angiogenesis. This work provides a novel system for the safe and efficient use of Bev and DOX on Neuroblastoma and explores the mechanism of the function of nano carrier in cancer therapy both in vitro and in vivo.

1. Introduction

Neuroblastoma, the most common pediatric malignant tumor, has a high ratio of mortality and morbidity[1]. Neuroblastoma in

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children over 1 year old usually show the symptom of metastasis [1]. Neither traditional chemotherapy nor radiation-therapy have an unsatisfied curative effect due to their high toxicity and risk of secondary malignancy [2–4]. The overall five-year survival in NB is only about 45% despite various treatments [5]. High curative chemotherapy drugs (such as DOX) for neuroblastoma have unexpected toxicity to normal tissues [6,7], therefore it is necessary to design a novel, non-toxic and targeted therapeutic strategy.

Nowadays drug-loaded nanocarrier has been considered as a promising method to reduce the poisonousness and improve therapy efficiency by increasing drug bioavailability and drug accumulation in tumor sites [8,9]. Over past decades, many kinds of nanocarriers were developed based on their excellent delivering characteristics. Inorganic nanoparticles, such as layered double hydroxide (LDH) and mesoporous silica nanoparticles (MSNs), have been commonly used as promising carriers for anti-cancer drugs and vaccines due to their satisfying biocompatibility, high specific surface, pH-sensitive and stimuli responsive release [10–18]. However, most nanocarriers do not have the active targeting ability for the special tumor. They are uptaken by cells mostly via the enhanced permeability retention (EPR) effect [19–25] so that native nanoparticles exhibit less accumulation within tumor cells than positive targeting ligands modified nanoparticles.

Vascular endothelial growth factor(VEGF) is one type of growth factors which could enhance cell invasion and new vessels formation through the effect on VEGF-VEGFR cascade signaling pathway. [26–31] Tumor angiogenesis could supply nutrients and oxygen to tumor tissue and remove wastes from the microenvironment. As a result, early cancers could be transformed to invasion and metastatic form [32,33]. According to many studies, VEGF is usually overexpressed in multiple solid tumor cells including neuroblastoma [1,34]. Hence, VEGF is recognized as an ideal target for anti-angiogenesis intervention. Bevacizumab (Bev, Avastin) is a humanized anti-VEGF antibody which was approved by the United States Food and Drug Administration on February 26, 2004 [27]. It specifically binds to VEGF forming a protein complex and then prevent VEGF from interacting with vascular endothelial growth factor receptor(VEGFR) [35]. This efficiently reduces the amount of available VEGF to play a role in angiogenesis. Numerous studies have demonstrated that Bev could perform an encouraging effect on a wide range of tumors such as brain, lung and breast cancer [36]. In clinical studies, Bev has exhibited significant therapeutic effects on many cancers combined with chemical drugs [36].

Herein, we developed a novel nano-composite for the targeting treatment of neuroblastoma and evaluated its performance. Firstly, in order to reduce the effect of the morphology of traditional LDH on practical applications [37], we synthesized a core-shell sphere structure LDH based on MSN(SiO_2@LDH) using layer by layer deposition. This structure enhanced cytophagy efficiency significantly and provide the pH-responsive ability to mesoporous silica core as reported previously [38]. Secondly, we modified SiO_2@LDH with Bev and had it loaded with DOX to form a new tumor vasculature targeting nano-composite, named as SiO_2@LDH-Bev-DOX. Finally, we explored its VEGF targeting ability, anti-angiogenesis and anti-tumor efficacy of neuroblastoma both in vitro and in vivo. In addition, the mechanisms underlying the anti-angiogenesis and anti-carcinoma efficiency were further investigated.

2. Materials and methods

2.1. Materials

Doxorubicin Hydrochloride (DOX) (purity $\geq$ 98.0%) was purchased from Hualan Chemical Technology Co.Ltd. (Shanghai China). Tetraethyl orthosilicate (TEOS), aluminum isopropoxide, 1,3,5-trimethylbenzene (TMB), 3-aminopropyltriethoxysilane (APTES), N-hydroxysuccinimide (NHS) and N-(3-dimethylamino propyl)-N-ethylcarbodiimide hydrochloride (EDC) were obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Cetyltrimethyl ammonium bromide (CTAB), NaOH, $Mg(NO_3)_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$, HCl (36%–38%), HNO_3 (69.2%), carbamide, methanol, ethanol and dimethyl sulfoxide (DMSO) were bought from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). Bevacizumab (Avastin) was acquired from Roche (Basel, Switzerland). Angiogenesis signaling pathway antibody kit (p-VEGFR2 (Tyr1175), p-FAK (Tyr397), p-Akt (ser473), p-Src (Tyr416), p-PLCγ1 (ser1248), p-p42/44MAPK (Erk1/2, Thr202/Tyr204), p-p38MAPK (Thr180/Tyr182), Horseradish peroxidase conjugated goat anti-rabbit antibody) and β-actin were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Water utilized for all experiments was of Millipore grade.

2.2. Nanoparticles preparations

2.2.1. Synthesis of MSNs

MSNs were synthesized by the soft template method which was introduced in the literature. In simple term, 0.985 g CTAB was dissolved in 100 mL methanol followed by 120 mL deionized water was added, then 0.57 mL NaOH (1 M) was injected into the mixed solution and 0.325 mL TEOS was added drop by drop. The resulting mixture was stirred at room temperature for 8 h. The products were collected by centrifugation and washed with water and ethanol to remove the residual reactants. The precipitants were resuspended by ethanol, then mixed with water and TMB for hydrothermal treatment at 140 °C for 4 days. After the pellet was washed with water and ethanol, the final products were refluxed by the mixture containing HCl and ethanol (v/v, 1:5) for 6 h at 85 °C to remove CTAB template.

2.2.2. Preparation of SiO_2@AlOOH nanoparticles

Based on the method reported, the AlOOH primer Sol was prepared. 11.3 g aluminum isopropoxide was absolutely dissolved by stirring at 85 °C in 100 ml deionized water. 1 M HNO_3 was then used to adjust the pH of aluminum isopropoxide solution from 3–4 to initiate the hydrolysis of aluminum isopropoxide. After the mixture was stirred at 85 °C for 2 h, AlOOH crystal would be acquired after evaporation. 5.8 g AlOOH crystal was dissolved in 107 ml deionized water, then 9 mL HNO_3 (1 M) was added to the solution, and the mixture was refluxed for 6 h accompanied with stirring at 85 °C to form AlOOH primer Sol. After that, SiO_2 nanoparticles were dispersed in AlOOH primer Sol for 1 h with vigorous stir. The ultimate resultant was washed with ethanol and water alternately and dried in a vacuum oven at 60 °C. The complete process including dispersion, collecting and drying were repeated 6 times.

2.2.3. Preparation of SiO_2@LDH nanoparticles

1.25 g of $Mg(NO_3)_2{\cdot}6H_2O$ and 2.4 g of NH_4NO_3 were dissolved in 60 mL deionized water. SiO_2@AlOOH nanoparticles were resuspended in the above-mixed solution and treated by hydrothermal treatment at 80 °C for 24 h. After being washed with water and ethanol, they were dried by freezing.

2.2.4. Synthesis of amine modified SiO_2@LDH

This process was carried out to link Bev with SiO_2@LDH. 60 mg SiO_2@LDH was dispersed in 150 mL isopropanol, 100 μl APTES was added in, then the mixture was refluxed with stirring for 3 h at 70 °C. The products were collected by centrifugation and washed with ethanol, the final SiO_2@LDH-NH_2 was preserved in MES (0.01 M, pH 6.5).

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