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Poly(D,L-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anticancer drugs

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Abstract

This research developed a novel bioadhesive drug delivery system, poly(D,L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) nanoparticles, for oral delivery of paclitaxel. Paclitaxel-loaded PLGA/MMT nanoparticles were prepared by the emulsion/solvent evaporation method. MMT was incorporated in the formulation as a matrix material component, which also plays the role of a co-emulsifier in the nanoparticle preparation process. Paclitaxel-loaded PLGA/MMT nanoparticles were found to be of spherical shape with a mean size of around 310 nm and polydispersity of less than 0.150. Adding MMT component to the matrix material appears to have little influence on the particles size and the drug encapsulation efficiency. The drug release pattern was found biphasic with an initial burst followed by a slow, sustained release, which was not remarkably affected by the MMT component. Cellular uptake of the fluorescent coumarin 6-loaded PLGA/MMT nanoparticles showed that MMT enhanced the cellular uptake efficiency of the pure PLGA nanoparticles by 57–177% for Caco-2 cells and 11–55% for HT-29 cells, which was dependent on the amount of MMT and the particle concentration in incubation. Such a novel formulation is expected to possess extended residence time in the gastrointestinal (GI) tract, which promotes oral delivery of paclitaxel.

Keywords: Anticancer drugs; Biodegradable polymers; Chemotherapy; Controlled release; Drug formulation; Medical clay

1. Introduction

Oral chemotherapy represents great challenges in drug delivery and its success could become a revolution in the history of chemotherapy. Oral chemotherapy can maintain an appropriate concentration of the drug in the circulation to achieve a prolonged exposure of cancerous cells to the drug. This will increase the efficacy and decrease the side effects of the anticancer drugs. The quality of life of the patients can be greatly improved. Unfortunately, most anticancer drugs such as paclitaxel are not orally bioavailable, i.e., not absorbable in the gastrointestinal (GI) tract. The oral bioavailability of paclitaxel was found to be less than 1% [1,2]. This is because the drug would be eliminated by the first-pass extraction of the cytochrome P450-dependent metabolic process and the overexpression of the multidrug efflux pump transporter P-glycoprotein (P-gp), which is rich in the intestine, liver, and kidney. An excellent work using wild-type and P-gp knockout mice showed the role of P-gp in multidrug resistance for poor oral bioavailability of paclitaxel [3]. Possible solutions for oral delivery of paclitaxel and other anticancer drugs are currently under intensive investigation. The general idea is to apply P-gp/P450 inhibitors such as cyclosporin A to suppress the elimination process [4–7]. P-gp/P450 inhibitors, however, suppress the body's immune system and thus cause medical complication. Moreover, most

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of the P-gp/P450 inhibitors have side effects and difficulties in formulation of their own. Nanoparticles of biodegradable polymers represent a chemotherapeutic engineering solution. It may provide a better solution for small-enough size and oral chemotherapy [8]. A typical example is the application of polymeric nanoparticles with appropriate surface coating to improve the adhesion and absorption of the nanoparticles to the intestinal cells and to escape from the recognition of P-gp [9–12]. High therapeutic efficacy and less side effects of the drug can thus be achieved. Moreover, the encapsulated drug can be released from the nanoparticles in a controlled and targeted manner. Desired pharmacokinetics can be achieved from specific design to meet various needs of individual patients.

In this research, we propose a novel nanoparticle formulation, i.e. biodegradable poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles incorporated with a medical clay, montmorillonite (MMT) (called PLGA/MMT nanoparticles hereinafter), for oral chemotherapy by using paclitaxel as a prototype drug due to its excellent therapeutic effects against a wide spectrum of cancers and its great commercial success as the best seller among various anticancer agents. MMT can provide mucoadhesive capability for the nanoparticle to cross the GI barrier [13]. MMT is also a potent detoxifier, which belongs to the structural family known as the 2:1 phyllosilicate. MMT could adsorb dietary toxins, bacterial toxins associated with gastrointestinal disturbance, hydrogen ions in acidosis, and metabolic toxins such as steroidal metabolites associated with pregnancy. All these conditions result in a host of common symptoms, including nausea, vomiting, and diarrhea, most of which are typical symptoms of the side effects caused by anticancer drugs. Calcium MMT has also been used extensively in the treatment of pain, open wounds, colitis, diarrhea, hemorrhoids, stomach ulcers, intestinal problems, acne, anemia, and a variety of other health issues. Not only does MMT cure minor problems such as diarrhea and constipation through local application, it also acts on all organs as well. Everything unhealthy, that emits negative radiations is irresistibly attracted to the medical clay and becomes subject to immediate elimination [14–21]. Our novel PLGA/MMT nanoparticle drug delivery system thus represents a new concept in developing drug delivery systems, formulating the drug carrier from a material, which can also have therapeutic effects, either synergistic with, or capable to mediate the side effects of the encapsulated drug.

Paclitaxel-loaded PLGA/MMT nanoparticles were prepared in this research by the emulsion/solvent evaporation method with MMT incorporated in the aqueous phase as a co-emulsifier in the fabrication process. The particle size and zeta potential were measured by the particle sizer and the zeta potential analyzer, respectively. The morphology of the drug-loaded nanoparticles was visualized by the scanning electron microscopy (SEM) and the atomic force microscopy (AFM). Physical status of the drug and MMT in the PLGA/MMT nanoparticles was investigated by differential scanning calorimetry (DSC) and X-ray diffraction (XRD), respectively. The content of MMT was estimated by thermal gravity analysis (TGA). High performance liquid chromatography (HPLC) was used to measure the drug encapsulation efficiency (EE) in the nanoparticles and to investigate the in vitro drug release kinetics. Cellular uptake of the fluorescent nanoparticles was quantified in two human colon derived cell lines, i.e. Caco-2 and HT-29 cells, by fluorescence spectroscopy. It was shown that MMT can significantly enhance the cellular uptake of the nanoparticles by Caco-2 and HT-29 cells and may thus have great potential for oral delivery of paclitaxel and other anticancer drugs.

2. Materials and methods

2.1. Materials

PLGA (50:50) and polyvinyl alcohol (PVA, Av. Mw 30,000–70,000) were purchased from Sigma. Paclitaxel of purity 99.8% was purchased from Dabur India Ltd. (India). Sodium MMT (Closite Na^+) was from Southern Clay Products Incorporation, USA. Dichloromethane (DCM), HPLC grade acetonitrile and Fluorescence marker coumarin-6 were from Aldrich.

2.2. Preparation of paclitaxel-loaded or coumarin 6-loaded PLGA/MMT nanoparticles

Paclitaxel-loaded PLGA/MMT nanoparticles were prepared by the emulsion/solvent evaporation method. In short, 5 mg paclitaxel and 110 mg PLGA were dissolved in 8 ml DCM. The resulting solution was emulsified in 120 ml aqueous solution containing 2% w/v PVA and various amounts of MMT (0, 0.046%, and 0.092% w/v) and then sonicated 120 s with the output power of 30 W. The formed emulsion was allowed to evaporate overnight at room temperature to harden the particles. The suspension was centrifuged, washed three times with deionized water and freeze-dried. Fluorescent coumarin 6-loaded PLGA/MMT nanoparticles were prepared in the same way except for that 0.5% (w/v) coumarin 6 was encapsulated instead of paclitaxel. Hereinafter PLGA/MMT nanoparticles prepared from 0, 0.046%, and 0.092% (w/v) MMT were termed NP0, NP1, and NP2, respectively.