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Development and evaluation of decorated aceclofenac nanocrystals

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ABSTRACT

This study was aimed at achieving enhanced solubility of aceclofenac (ACF) in nanocrystaline forms (ACF-NC) and evaluating the effects of ACF-NC on cell viability. Decorated ACF-NC were prepared by nano-precipitation with stabilizers. Three kinds of stabilizers were investigated: Tween 80, Poloxamer 407, and PEG 6000. The crystal structure and morphology of ACF-NC were characterized by field emission scanning electron microscopy (FE-SEM) and differential scanning calorimetry (DSC). The solubility of ACF-NC and ACF (pure) was evaluated in different media (pH 1.2 and pH 6.8 buffers and distilled water [DW]). A drug release study was performed in PBS for 24 h. Cell viability was evaluated for 24 h using a human colon cancer cell-line (HCT-116) and a human breast cancer cell-line (MCF-7).

Decorated ACF-NC with a mean size of 725 nm were successfully prepared. The solubility of the decorated ACF-NC were 4–7 times higher than that of ACF in DW and pH 6.8 buffer. A peak shift from 153.1 °C to 150.5–151.0 °C was observed in the DSC thermogram of decorated ACF-NC versus ACF. In terms of drug release, there was an initial burst in decorated ACF-NC within 1 h followed by slow release for up to 4 h. Decorated ACF-NC exhibited viability approximately 63.9% of HCT-116 cells and also showed viability in 58.3% of MCF-7 cells at 15 μg/mL of drug concentration. In conclusion, decorated ACF-NC proved to be a promising approach for enhancing drug solubility and cytotoxicity.

1. Introduction

According to the Biopharmaceutics Classification System (BCS II), aceclofenac (ACF) is a non-steroidal anti-inflammatory drug (NSAID) with poor water solubility (0.058 μg/mL) and high permeability [1] (Fig. 1). Despite the high permeability of the BCS class II drugs, they often result in low oral bioavailability due to their slow and limited release of drug in gastrointestinal fluid [2]. The market of ACF is limited to Asia, therefore, attempts to develop ACF formulations with high efficacy have to be made in order to expand its market.

Approximately 40% of drugs in the development pipelines are poorly soluble in aqueous solvents and some of them in the organic solvents as well [3]. Low aqueous solubility and dissolution rate of API is one of the most prevalent problem that formulation scientists are facing, and it is more common among the new drug candidates due to the use of high throughput and combinatorial screening processes during the drug discovery and selection of the new molecular entity (NME) [4,5]. Poorly water-soluble drugs are difficult to develop with commercially available techniques and are frequently abandoned early in the drug development pipeline. The main goal of formulation development for these drugs is to improve solubility. To achieve solubility enhancement, several techniques have been developed, such as solid dispersion [1,6], salt formation [7], co-solvent [8] and particle size reduction (micronization and nanoparticle formation) [9,10]. However, often they cannot solve the bioavailability problem for many drugs. For example, micronization does not create the sufficiently large surface to enhance dissolution rates and therefore, the bioavailability of the many poorly soluble drugs.

To overcome the challenge of poor solubility, nanomaterials such as nanocrystals, solid lipid nanoparticles (SLN), nanoemulsions, and self-assembled nanoparticles have been employed to enhance drug solubilization [11]. Among the various nanotechniques, drug nanocrystal formulation has been successfully explored for delivery of various anti-cancer drugs [12–15]. Nanonization of these drug molecules have increased the solubility and dissolution rate owing to increased surface area [16]. Drug nanocrystals ideally exist in a stable crystalline state with high

Abbreviations: ACF, aceclofenac; NC, nanocrystals; ACF-NC, aceclofenac nanocrystals; FE-SEM, field emission scanning electron microscopy; DSC, differential scanning calorimetry; DW, distilled water.

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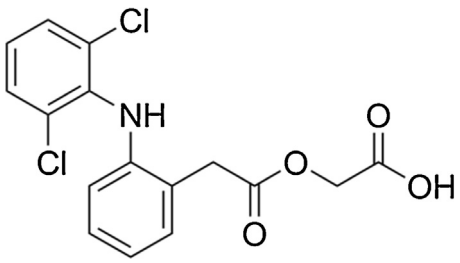

Fig. 1. Chemical structure of Aceclofenac.

drug loading which can be formulated as the oral dosage dorms with improved dose-bioavailability, proportionality and increased patient compliance, and can also be injected intravenously as the aqueous nanosuspensions [17]. Drug nanocrystals can be produced mainly by applying two approaches, top-down and bottom-up [18,19], which utilize different techniques. Top-down approaches utilize high-energy processes whereby pure drugs are broken down into smaller-sized particles by the use of technologies such as pearl milling and high-pressure homogenization [20–22]. Products on the pharmaceutical market such as Emend®, Tricor®, Tridlide®, Naprelan®, and Theodur® were produced by the top-down approach utilizing milling technology. Bottom-up approach comprises of the precipitation of the dissolved drug by a non-solvent and controlled crystallization during freeze drying [23]. The bottom-up approach was applied for the development of products such as Gris-Peg® and Cesamet® [18].

In this study, nanocrystals were prepared by a nanoprecipitation method to reduce the particle size of the drug, thereby enhancing the surface area for solubilization and cellular viability. Decorated ACF nanocrystals (ACF-NC) were evaluated with respect to physical properties of morphology, solubility, and drug release. In addition, the effects of ACF and decorated ACF on cell viability were investigated in colon and breast cancer cell lines.

2. Materials and methods

2.1. Materials

ACF was obtained from Korea United Pharm., INC. Tetrazolium dye 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma–Aldrich (Saint Louis, MO, USA). HCT-116 cells and MCF-7 cells were obtained from the College of Pharmacy, Chosun University. Fetal bovine serum (FBS), antibiotics, and Roswell Park Memorial Institute (RPMI1640) medium were from Gibco (Billings, MT, U.S.A.). Organic solvents were purchased from Samchun Pure Chemicals.

2.2. Preparation of decorated ACF-NC

ACF-NC were obtained by the following nanoprecipitation method. ACF (20 mg) was dissolved in acetone (5 mL), in which 20 mg of the polymers, i.e., Tween80, Poloxamer 407, and PEG-6000 were subsequently dissolved as stabilizers. The resulting ACF and polymer-containing solutions were added to an aqueous medium. Size of the drug crystals were controlled by probe-sonication (Sonics & Materials INC, VCX 500, USA) at 200 W for 1 min while stirring at 300 rpm. The nanocrystals were collected by filtration with a nylon filter (0.2 μm pore size) and washed two times with DW. The nanocrystals were first dried in a drying oven at 60 °C for 1 h, followed by further drying in a desiccator under vacuum for 1 day.

2.3. Characterization of decorated ACF-NC

2.3.1. Particle size analysis

Morphological evaluations of ACF-NC were conducted by scanning electron microscopy (FE-SEM, JSM-6700F, JEOL, Japan). ACF and ACF-NC powders were dropped onto carbon tape. The carbon tape was coated with gold for 2 min under vacuum. Samples were viewed at an acceleration voltage of 5.0 kV, and the sizes of at least 50 particles were measured manually using ImageJ software (National Institute of Health).

2.3.2. Differential scanning calorimetry (DSC)

Thermographs of the decorated ACF-NC and ACF samples were obtained by using a differential scanning calorimetry (DSC) instrument (DSC 200 F3, NETZSCH, Germany). Samples equivalent to 1 mg of ACF were placed in aluminum pans and heated from 10 °C to 250 °C at a scanning rate of 10 °C/min under a nitrogen purge of 40 mL/min.

2.3.3. Solubility test

The solubility of decorated ACF-NC and ACF samples was determined by using a water bath shaker (BS-06, Lab Company, Korea). ACF powder (5 mg) and ACF-NC (equivalent to 5 mg ACF) were dispersed in 5 mL of pH 1.2 buffer, pH 6.8 buffer, and D.W, followed by shaking at 100 rpm at 37 °C. After 1 h, the samples were subjected to centrifugation at 20,000*g* for 30 min and then filtered through a PVDF syringe filter (0.22 μm pore size). The ACF content of the sample was determined using high-performance liquid chromatography (HPLC).

2.3.4. HPLC analysis

ACF for solubility analysis was subjected to HPLC (Waters 2695 Alliance system, Waters, USA) with an ultraviolet detector (Waters 2484, Waters, USA) set at a wavelength of 275 nm. A C18 column (Gemini 5u 110A analytical, Intersil OSD-3) operated at 30 °C was used as the analytical column The mobile phase was composed of methanol–0.02% orthophosphoric acid (70:30) at a flow rate of 1 mL/min [24].

2.3.5. Drug release

In vitro ACF release studies were performed in pH 7.4 phosphate-buffered saline (PBS) at 48 h. The shaking speed was set to 100 rpm. Samples (1 mL) were withdrawn at 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h. The samples were subjected to centrifugation at 20,000*g* for 30 min and then filtered through a PVDF syringe filter (0.22 μm pore size). The ACF content of the sample was determined using high performance liquid chromatography (HPLC).

2.3.6. Cell viability

Human colon cancer cells (HCT-116) and human breast cancer cells (MCF-7) were grown in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum and (5%) antibiotics (100 IU/mL of penicillin G sodium and 100 μg/mL of streptomycin sulfate). Cells were maintained in an incubator under a humidified 5% CO_2/95% air atmosphere at 37 °C. They were seeded in 96-well plates at a density of 3×10^4 cells/well and grown to 70–80% confluence. ACF and decorated ACF-NC equivalent to an ACF concentration of 0.15, 1.5 and 15 μg/mL were added and cells were incubated for 12 and 24 h. Cytotoxicity was assayed using MTT and detected using a microplate reader (Synergy H1Hybrid Multi-Mode Microplate Reader, BioTek, USA). The cytotoxicity percentage was calculated by subtracting% cell viability from 100%.

2.3.7. Statistical analysis

Statistical analysis was performed using the paired *t*-test in SigmaPlot 10.0 (SYSTAT, Inc., Chicago, IL, USA). The data were