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Development of nanocrystal formulation of meloxicam with improved dissolution and pharmacokinetic behaviors

Masanori Ochi [a,*], Takaki Kawachi [a], Eri Toita [a], Issei Hashimoto [a], Kayo Yuminoki [a], Satomi Onoue [b], Naofumi Hashimoto [a]

[a] Department of Pharmaceutical Physicochemistry, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan
[b] Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-Ku, Shizuoka 422-8526, Japan

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ABSTRACT

The present study aimed to develop nanocrystal formulations of meloxicam (MEL) in order to enhance its biopharmaceutical properties and provide a rapid onset of action. Nanocrystal formulations were prepared by wet-milling and lyophilization with hydrophilic polymers used as aggregation inhibitors. Aggregation inhibitors were selected based on high-throughput screening of crystal growth inhibition in supersaturated MEL solution. Supersaturation of MEL was observed in PVP K-30, HPC-SSL, and POVACOAT Type F solution. Although the particle size distributions of pulverized MEL with PVP K-30 (MEL/PVP), HPC-SSL (MEL/HPC), and POVACOAT Type F (MEL/POVA) were in the nanometer range following lyophilization, increases in micron-sized aggregates were observed after storage at 60 °C for 21 days. The order of increased amount of aggregates was MEL/POVA ≫ MEL/HPC > MEL/PVP. These findings showed that hydrophilic polymers that inhibited crystal growth in supersaturated MEL solutions tended to prevent aggregation. The dissolution behavior of all nanocrystal formulations tested was markedly enhanced compared with that of unpulverized MEL. Oral administration of MEL/PVP showed a 2.0 h shortened T_{max} and a 5.0-fold increase in bioavailability compared with unpulverized MEL. These findings showed that the MEL/PVP mixture was physicochemically stable and provided a rapid onset of action and enhanced bioavailability after oral administration.

1. Introduction

Meloxicam (MEL) is a highly potent non-steroidal anti-inflammatory drug (NSAID) used to treat rheumatoid arthritis (Furst, 1997), osteoarthritis (Hosie et al., 1996), and postoperative pain (Aoki et al., 2006). MEL selectively inhibits cyclooxygenase-2 isoenzyme (Pairet et al., 1998), consequently, MEL has effective anti-inflammatory and analgesic properties and low gastrointestinal toxicity. Despite these attractive pharmacological profiles, the onset of the pharmaceutical effects of MEL is slow due to slow oral absorption. A rapid onset of pharmaceutical effects is important to patients, particularly during acute exacerbation of rheumatism and osteoarthritis. Poor wettability and low solubility of MEL (ca. 0.6 μg/mL at both pH 1.2 and 4.0) (Ghorab et al., 2004) are believed to be as responsible for the slow oral absorption. Consequently, the application of solubilization technologies could be a key for improving the pharmacological profile of MEL.

A number of approaches have been made to enhance the aqueous solubility of poorly soluble drugs include salt formation (Serajuddin, 2007), nano-pulverization (Merisko-Liversidge et al., 2003), emulsification (He et al., 2010) and amorphous solid dispersion technique (Leuner and Dressman, 2000). Nano-pulverization is one of the most simple and effective methods for improving the dissolution behavior and bioavailability of drugs. However, well-known problems associated with nanocrystal formulations include increased aggregation during preparation and storage (Abdelwahed et al., 2006; Quan et al., 2012). Increased aggregation leads to a reduction in surface area, causing a reduction in dissolution rate and systemic exposure. Therefore, the selection of an appropriate aggregation inhibitor is very important for the development of nanocrystal formulations. It has been reported that nanoparticles can be stabilized by covering the surface of the drug nanoparticles with suitable polymers (Peltonen and Hirvonen, 2010). Appropriate intermolecular interactions between the drug and polymer molecules on the surface of the nanocrystal are believed to be important for

Abbreviations: HPC, hydroxypropyl cellulose; MEL, meloxicam; POVA, POVACOAT Type F; PVP, polyvinylpyrrolidone; XRPD, X-ray powder diffraction.

* Corresponding author. Tel.: +81 72 866 3153; fax: +81 72 866 3153.
E-mail addresses: 12d403om@edu.setsunan.ac.jp, d09185@gmail.com (M. Ochi).

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preventing aggregation. Chauhan et al. previously demonstrated that the crystal growth inhibition efficiency of a supersaturated drug solution correlated well with the stability of the amorphous solid dispersion (Chauhan et al., 2013). Screening polymers for their MEL crystal growth inhibition might help narrow down the number of aggregation inhibitor candidates for improving MEL nanocrystal formulations. However, to date, there has been no effort to screen crystal growth inhibitors for improving nanocrystal formulations. In this study, a 96-well filter-plate-based anti-precipitant screening approach was used because of its simplicity and applicability to automated high-throughput analysis (Yamashita et al., 2011).

This study aimed to develop a physicochemically-stable MEL nanocrystal formulation with improved dissolution behavior in order to shorten the onset of pharmacological effects. The physicochemical properties were characterized by X-ray powder diffraction (XRPD), particle size distribution analysis before and after storage using a laser diffraction/scattering method, and dissolution testing in acidic and neutral media. The physical stability and dissolution profile results suggested the most suitable MEL nanocrystal formulation for in vivo testing. Pharmacokinetic profiling of MEL after oral administration of the nanocrystal formulation to rats was evaluated.

2. Materials and method

2.1. Materials

Crystalline MEL was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Hydroxypropyl cellulose (HPC) was obtained from Nippon Soda Co., Ltd. (Tokyo, Japan). Polyvinylpyrrolidone (PVP) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Eudragit E PO ((poly(butyl methacrylate-*co*-(2-dimethylaminoethyl) methacrylate-*co*-methyl methacrylate) 1:2:1), Eudragit L 100 (poly(methacylic acid-*co*-methyl methacrylate) 1:1) and Eudragit RL PO (poly(ethyl acrylate-*co*-methyl methacrylate-*co*-trimethylammonioethyl methacrylate chloride) 1:2:0.2) were provided by Evonik Industries (Darmstadt, Germany). POVACOAT Type F (polyvinyl alcohol/acrylic acid/methyl methacrylate copolymer) was kindly supplied by Daido Chemical Co., Ltd. (Kobe, Japan). Hydroxypropylmethylcellulose acetate succinate (HPMC-AS) was provided by Shin-Etsu Chemical (Tokyo, Japan). Acetonitrile (liquid chromatography grade) was purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were purchased from commercial sources.

2.2. HPLC/UV analysis

The concentration of MEL was determined by an absolute calibration method using a high-performance liquid chromatography (HPLC) system equipped with a diode array detector (Shimadzu, Kyoto, Japan). The HPLC system consisted of a LC-20A solvent delivery unit with high-pressure flow-line selection valves, a SIL-20A auto sampler, a CTO-20A column oven, and a SPD-M20A diode array detector connected to the LC solution software. Chromatography was conducted using a COSMOSIL $5C_{18}$-AR-II column (particle size: 5 μm, column dimensions: 4.6 mm × 150 mm). Column temperature was maintained at 40 °C, and the samples were separated using a mobile phase consisting of 20 mM phosphate buffer (pH 7.0) and acetonitrile (3/7, v/v) at a flow rate of 0.5 mL/min. The diode array detector was set at 360 nm.

2.3. Anti-precipitant screening by the solvent shift method

Polymers were dissolved or homogeneous-dispersed in distilled water at 150 μg/mL. Hydrophilic polymer solution or suspension (200 μL) was placed in each well of a 96-well filter plate (MultiScreen HTS solubility filter plate, 0.45 μm, Millipore). Then, 1 μL of 30 mg-MEL/mL solubilized in DMSO was added to each well using a pipette, then the plate was shaken in an IKA MS 3 digital MIXER (IKA, Staufen, Germany) for 1 h at 25 °C. The samples were centrifuged at 500 × *g* for 5 min, and the filtrates were collected in a 96-well plate. The filtrates were diluted with an equivalent volume of acetonitrile, then the concentration of MEL in each sample was measured using HPLC/UV.

2.4. Wet-milled formulation of MEL

Nanocrystal formulations of MEL were prepared using a rotation/revolution mixer (NP-100; Thinky Company Ltd., Tokyo, Japan) as reported previously (Takatsuka et al., 2009). Briefly, 20 mg of MEL and 2.5 g of zirconia (zirconium oxide) balls (0.1 mm diameter; Nikkato Company, Ltd., Osaka, Japan) were weighed into the vessel of a rotation/revolution mixer, and 0.5 mL of hydrophilic polymer solution (40 mg/mL) was added. MEL suspension was nano-pulverized by two-step wet-milling as follows: the first step, 2000 rpm for 2 min with the polymer solution; the second step, 400 rpm for 1 min after the addition of 4.5 mL of distilled water. After nano-pulverization, each MEL suspension containing 20 mg of milled MEL and 20 mg of the polymer in a 10 mL vial was frozen in liquid nitrogen and freeze-dried using a FD-81 freeze dryer (Tokyo Rikakikai, Tokyo, Japan).

2.5. XRPD analysis

XRPD patterns were collected with a RINT diffractometer (Rigaku Co., Tokyo, Japan) with Cu Kα radiation generated at 40 mA and 40 kV. Data were obtained from 5° to 40° (2θ) at a step size of 0.2° and a scanning speed of 5°/min.

2.6. Particle size distribution

The particle size distribution and specific surface areas of the MEL samples in water were determined by a laser diffraction/scattering method using a Mastersizer 2000 equipped with a Hydro 2000 μP (Malvern Instruments Ltd., Worcestershire, UK). Volume percent of micron size aggregate was calculated using the total sum of volume percent of particles with particle size bigger than 1 μm. MEL sample (16 mg) was suspended in 2.0 mL of distilled water and dispersed gently without sonication. The particle size distribution was expressed as the volume median diameter and SPAN factor defined as $\mathrm{SPAN} = (d_{90} - d_{10})/d_{50}$, where d_{10},d_{50}, and d_{90} are the particle diameters at 10%, 50%, and 90% of the cumulative volume, respectively. A high SPAN value indicates a wide size distribution.

2.7. Particle size stability study

MEL samples were placed in sealed vials under 60 °C for 21 days, then were subjected to particle size distribution analysis.

2.8. Dissolution test

Dissolution tests were carried out in 900 mL of HCl solution (pH 1.2) using a Japanese Pharmacopeia dissolution apparatus I (NTR-VS6, Toyama Sangyo Co., Ltd., Osaka, Japan). The rotating basket method was used, with constant stirring at 100 rpm at 37 °C. Each MEL sample was weighed to keep the total amount of MEL in the dissolution vessel constant at 15 mg. 1 mL of samples were collected at 7.5, 15, 30, 60, 90 and 120 min using a pipette. It was not replaced. The sampling site was 1 cm from the test vessel wall at ca. 25 mm below the surface of the medium. Samples were centrifuged at 15,000 × *g* for 15 min to remove insoluble materials.