International Journal of Pharmaceutics 434 (2012) 148–154

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Novel formulations of dipyridamole with microenvironmental pH-modifiers for improved dissolution and bioavailability under hypochlorhydria

Chika Taniguchi[a,b], Ryo Inoue[a], Yohei Kawabata[a,b], Kazuhiro Yamashita[b], Koichi Wada[b], Yukinori Yamauchi[c], Shizuo Yamada[a], Satomi Onoue[a,*]

[a] Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Department of Chemistry, Manufacturing and Control, Kobe Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd., 6-7-5, Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan
[c] Department of Pharmaceutical Physical Chemistry, College of Pharmaceutical Sciences, Matsuyama University, 4-2, Bunkyo, Matsuyama, Ehime 790-8578, Japan

ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 18 April 2012
Accepted 19 May 2012
Available online xxx

Keywords:
Dipyridamole
pH-modifier
Dissolution
Bioavailability
Hypochlorhydric

ABSTRACT

This study was undertaken to develop new dipyridamole (DP) formulations with acidic microenvironmental pH-modifiers for improving dissolution and absorption under hypochlorhydric conditions. Dipyridamole granules (DPG) with ten acidic pH-modifiers were prepared with conventional wet granulation, and their manufacturability, stability and dissolution behavior were characterized. Pharmacokinetic profiling of the optimized DPG with acid was carried out in omeprazole-treated rats as a hypochlorhydric model. On the basis of the manufacturability, stability and dissolution behavior of new DPG formulations, *p*-toluenesulfonic acid (TS) was found to be a suitable acidic pH-modifier for DPG formulation. Although DPG showed pH-dependent dissolution behavior, DPG with TS exhibited a high rate and extent of dissolution in both acidic and neutral media. After oral administration of DPG (10 mg DP/kg) in omeprazole-treated hypochlorhydric rats, there was ca. 40% reduction of the area under the curve of plasma concentration vs. time from zero to 3 h (AUC_{0-3}) for DPG compared with that in normal rats. However, AUC_{0-3} for DPG/TS under hypochlorhydria was almost identical to that of DPG in normal rats. From these findings, the addition of TS as a microenvironmental pH-modifier in DP formulation might be beneficial in expanding the therapeutic potential of DP in hypochlorhydric patients.

1. Introduction

Dipyridamole[2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine] (DP), a thromboxane synthase inhibitor, has been clinically used for prevention of postoperative thromboembolic complication or reduction of the reoccurrence of transient ischemic attacks (Fukawa et al., 1982; Harker and Kadatz, 1983). DP is a weakly basic drug with a pK_a value of 6.4 (Kostewicz et al., 2002), and thereby, its solubility has a strong pH-dependent profile with high solubility in the acidic range up to pH 4, which drops by three orders of magnitude with the appearance of low solubility at above pH 4 (Zhou et al., 2005). DP was categorized as a biopharmaceutics classification system (BCS) class II drug (Butler and Dressman, 2010) because of its low solubility and high permeability. In general, weakly basic drugs sometimes exhibit poor oral bioavailability with wide variability, depending on the gastric condition of patients, especially hypochlorhydric patients or patients who commonly use proton pump inhibitors or H_2-blockers (Russell et al., 1994). DP showed low oral bioavailability in famotidine-treated patients with increased gastric pH, and the oral bioavailability of DP was reduced ca. 40% with hypochlorhydric subjects compared with that in control subjects (Russell et al., 1994).

To improve the bioavailability of DP, several formulations have been developed, which include a solid dispersion (Chen et al., 2007), dipyridamole/β-cyclodextrin complexation (Ricevuti et al., 1991) and nano-mixing formulation (Sanganwar and Gupta, 2009). However, these formulations and parts of their manufacturing processes are complicated, so it might be challenging to manufacture them industrially as commercial products. Recently, considerable

Abbreviations: ANOVA, analysis of variance; API, active pharmaceutical ingredient; AA, adipic acid; D, L-aspartic acid; AUC, area under the curve of plasma concentration vs. time; BCS, biopharmaceutics classification system; CA, citric acid monohydrate; C_{max}, maximum concentration; CV, coefficient of variation; DP, dipyridamole; DPG, dipyridamole granule; E, L-glutamic acid; HCl, hydrochloric acid; HPC, hydroxypropyl cellulose; HPLC, high-performance liquid chromatography; IDR, intrinsic dissolution rate; MLE, maleic acid; MLI, *dl*-malic acid; PK, pharmacokinetic; TS, *p*-toluenesulfonic acid monohydrate; RH, relative humidity; SA, succinic acid; SEM, scanning electron microscopy; SIR, selected ion recording; $T_{1/2}$, half-life; TA, L-tartaric acid; T_{max}, time to maximum concentration; UPLC/ESI-MS, ultra performance liquid chromatography equipped with electrospray ionization mass spectrometry; UV, ultraviolet; PXRD, powder X-ray diffraction.

* Corresponding author. Tel.: +81 54 264 5633; fax: +81 54 264 5635.
E-mail address: onoue@u-shizuoka-ken.ac.jp (S. Onoue).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.05.040

attention has also been drawn to the pH-modifier approach because of its simplified manufacturing processes and cost performance. To use an acid or base as a pH-modifier is to enhance dissolution behavior and bioavailability of a drug substance with pH-dependent solubility by decreasing or increasing microenvironmental pH (Badawy and Hussain, 2007). As a controlled release formulation, DP matrix tablet with acidic pH-modifier has already been developed (Siepe et al., 2006, 2008). In these previous studies, four acids, including fumaric acid (FA), succinic acid (SA), citric acid (CA) and ascorbic acid, were used as pH-modifiers in which the DP formulation with FA exhibited ca. 3-fold higher dissolution rate at pH 6.8 than the acid-free DP formulation. These four pH-modifiers were weak acids with pK_a values of above 3, and other strong acids have the possibility to act as more potent acidic pH-modifiers than these four acids used previously.

The objective of this study was to develop an immediate-releases formulation of DP with pH-modifier for enhancing bioavailability and dissolution behavior. In the present study, granule formulations of DP (DPG) with seven acidic pH-modifiers were newly prepared with conventional wet granulation. Selection of appropriate acid and formulation optimization were carried out with a focus on manufacturability, stability (chemical/photo) and dissolution behavior. Pharmacokinetic study on the optimized DP formulation was also performed to clarify the possible improvement in oral bioavailability using omeprazole-treated rat as a hypochlorhydric model.

2. Materials and methods

2.1. Chemicals

Dipyridamole (DP) was produced by Boehringer Ingelheim GmbH (Ingelheim, Germany), and the specification tests were carried out according to the Japanese pharmacopeia (15th edition). Mannitol was purchased from Roquette GmbH (Frankfurt, Germany). Hydroxypropyl cellulose (HPC) was purchased from IMCD Deutschland GmbH & Co. KG (Cologne, Germany). L-Tartaric acid (TA) was purchased from Tartarica Treviso S.R.L. (Villorba, Italy). Citric acid monohydrate (CA) was purchased from Jungbunzlauer Ladenburg GmbH (Ladenburg, Germany). Fumaric acid (FA) was purchased from Bartek Ingredients Inc. (Ontario, Canada). *p*-Toluenesulfonic acid monohydrate (TS) and maleic acid (MLE) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Adipic acid (AA) was provided by Asahi Kasei Corporation (Tokyo, Japan). *dl*-Malic acid (MLI) was provided by Showa Kako Corporation (Osaka, Japan). Succinic acid (SA) was provided by Kawasaki Kasei Chemicals Ltd. (Kawasaki, Japan). L-Aspartic acid (D) and L-glutamic acid (E) were provided by Kyowa Hakkou Bio Co., Ltd. (Tokyo, Japan). All other chemicals were purchased from commercial sources.

2.2. Wet granulation of dipyridamole

2.2.1. Preparation

DP granule (DPG) and DPG with acidic pH-modifier were prepared with conventional wet granulation. DP, mannitol and acid were mixed, 5% HPC solution was added into the mixture and granulated with mortar and pestle. The wet granule was dried at 60 °C using a vacuum drying oven, DP23 (Yamato Scientific Co., Ltd., Tokyo, Japan) for 2 h. The dried granule was passed through a 1 mm-mesh screen. Drug load in the composition was 30% of the total amount and the acid amount was the same as the drug substance for the acid selection study. For acid amount optimization study, drug load was also 30% and acid load was changed from 15% to 60% of the total composition.

2.2.2. Scanning electron microscopy (SEM)

Representative SEM images of DPG or DPG with acid were taken using a scanning electron microscope, VE-7800 (Keyence Corporation, Osaka, Japan), without Au or Pt coating. For the SEM observations, each sample was fixed on an aluminum sample holder using double-sided carbon tape.

2.2.3. Dipyridamole determination

The amount of DP in the obtained granule was determined using the HPLC system with UV detection at 410 nm, Waters Alliance 2695 with Dualλ absorbance detector 2487 (Waters Corporation, Milford, MA, USA). An ODS column (particle size: 5 μm, column size: 3.0 mm × 60 mm; Inertsil ODS-2, GL Sciences, Inc., Torrance, CA, USA) was used and column temperature was maintained at 40 °C. Samples were separated using an isocratic mobile phase consisting of the mixture of 0.48 M ammonium formate buffer (pH 6.5), methanol and acetonitrile (580:240:180) with a flow rate of 1.0 mL/min, and the retention time of DP was ca. 15 min. Purity was calculated against standard solution.

$$\text{Purity}\ \% = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{concentration of standard solution}}{\text{concentration of sample solution}} \times \text{potency of standard}$$

2.3. Stability tests

2.3.1. Stress stability study

For stress stability study, about 1 g of each DP granule was poured into a 25 mL brown color glass bottle. The samples were stored at 40 ± 2 °C/75 ± 5% relative humidity (RH) in a stability chamber SRH-15VEVJ2 (Nagano Science Co. Ltd., Osaka, Japan) and 60 ± 2 °C in a stability chamber LH21-15M (Nagano Science Co. Ltd., Osaka, Japan) for 2 weeks and 4 weeks. After storage, the samples were evaluated to purity according to Section 2.2.3.

2.3.2. Photostability tests

For photostability testing, each granule containing 50 mg of DP was weighed exactly and spread in a 25 mL clear glass bottle. The samples were stored in the SUNTEST XLS+ (Atlas Material Technology LLC, Illinois, USA) and the amount of DP remaining in the granule was determined by HPLC as described in Section 2.2.3. The UVA/B and visible light irradiation was carried out at 25 °C with an irradiance of 250 W/m^2 in the wavelength range of 300–800 nm for 24 h.

2.4. Dissolution properties

2.4.1. Dissolution test

Dissolution tests were carried out for 12 h by the paddle method at 50 rpm in 900 mL of 0.05 M phosphate buffer (pH 6.8) and/or 0.1 M hydrochloric acid (HCl) solution (pH 1) using the dissolution tester system with UV automatic flow system, NTR-6100 (Toyama Sangyo Co., Ltd., Osaka, Japan) at 37 °C. For comparative study under supersaturated conditions, the granule was weighed to keep the total amount of DP in the dissolution vessel constant at 25 mg for acid selection and 50 mg for acid amount optimization, equal to ca. 4.6- and 9.3-times of the equilibrium solubility (Cs). Samples were measured at the indicated times with automatic ultraviolet (UV) flow cell at 298 nm for pH 6.8 buffer solution and at 283 nm for 0.1 M HCl solution. The C/Cs value as the super-saturation ratio was calculated from the actual DP concentration in the dissolution medium (C) and Cs.