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

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ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 18 April 2012
Accepted 19 May 2012
Available online 27 May 2012

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.

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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.

2.4.2. Intrinsic dissolution rate

The intrinsic dissolution rate (IDR) of DP was determined in aqueous media covering the range of pH 1.1–6.0 using the rotating disc method, which maintains a constant surface area. Five mg of drug substance was compressed to form a disc. These discs were mounted on a sample holder, which fits into a dissolution tester (Sotax, Basel, Switzerland). The dissolution media were stirred at 200 rpm. Samples were automatically withdrawn from the dissolution vessel and assayed by UV spectrophotometry. The IDR expressed in mg/cm^2/min was calculated using the slope of the concentration vs. time plot and from the linear portion of the slope of the dissolution curve, volume of dissolution medium (35 mL) and area (diameter: 2 mm) of the exposed disk.

2.5. Pharmacokinetics

2.5.1. Animals

Male Sprague–Dawley rats, weighing ca. 307 ± 38 g (8–9 weeks of age; Japan SLC, Shizuoka, Japan), were housed two per cage in the laboratory with free access to food and water, and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1 °C) and humidity (55 ± 5%). All procedures used in the present study were conducted in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka.

2.5.2. UPLC/ESI-MS analysis for plasma concentration of DP

Blood samples (200 μL) were collected from the tail vein at the indicated periods after intravenous administration of DP (3.0 mg/kg) dissolved in 0.2% tartaric acid (pH 2.4) or oral administration of 0.5 mL of DPG suspension or DPG/TS30 suspension (10 mg DP/kg). Suspensions of DPG and DPG/TS30 were immediately administered just after preparation of suspension. Each blood sample (200 μL) was centrifuged at 10,000 × *g* to prepare plasma samples. To 50 μL of plasma sample, 150 μL of methanol was added, and the solution was centrifuged at 3000 × *g* for 10 min. The supernatant was filtrated through a 0.2-μm filter, and then the filtrate was analyzed by an internal standard method using a Waters Acquity UPLC system (Waters, Milford, MA), which included binary solvent manager, sample manager, column compartment and SQD connected with MassLynx software. An Acquity UPLC BEH C 18 column (particle size: 1.7 μm, column size: 2.1 mm × 50 mm; Waters) was used, and the column temperature was maintained at 40 °C. The samples were separated using a gradient mobile phase consisting of acetonitrile (A) and 5 mM ammonium acetate (B) with a flow rate of 0.25 mL/min. The gradient conditions of mobile phase were 0–0.5 min, 40% A; 0.5–2.5 min, 40–95% A; 2.5–3.0 min, 95% A; and 3.0–3.5 min, 40% A. Analysis was carried out using selected ion recording (SIR) for specific *m*/*z* 429 and 505 for DP, and DP was detected at a retention time of 1.53.

2.6. Statistical analysis

For statistical comparisons, one-way analysis of variance (ANOVA) with pairwise comparison by Fisher's least significant difference procedure was used. A *P* value of less than 0.05 was considered significant for all analyses.

3. Results and discussion

3.1. Preparation and stability study on dipyridamole granule with pH-modifier

Dipyridamole granule (DPG) and DPG with pH-modifiers of ten acids were prepared, and FA, CA and SA, used in a previous study (Siepe et al., 2006), were included in these ten acids for comparison. The pH values for saturated solutions of these ten acids were below 4 (data not shown), and this characteristic might improve DP dissolution behavior by decreasing microenvironmental pH. Manufacturability of the DPG was different depending on the type of acid. DPG with TA, DPG with CA, DPG with MLI, DPG with MLE and DPG with TS were found to have a candy-like appearance just after wet granulation. Levels of solubility of TA, CA, MLI, MLE and TS in water were 206 mg/mL, 1330 mg/mL, 558 mg/mL, 790 mg/mL and 670 mg/mL, respectively (Stahl and Wermuth, 2011), so these five acids could be partly dissolved through contact with the granulation binder during wet granulation, leading to problematic preparation. According to SEM images (Fig. 1), DPG and DPG/TS were formed as fine granules ca. 25–50 μm in length. In contrast, DPG/TA was a larger granule ca. 200–300 μm in length, and its surface was partly melted. DPG/MLE was found to have a candy-like appearance even after drying, possibly due to high hygroscopicity. DPG with acidic pH-modifiers, except for DPG with MLE, were obtained successfully, and physicochemical properties of these formulations were characterized.

In order to confirm the influence of the addition of acidic pH-modifiers into DPG on chemical stability, stability testing on DPG and DPG with acidic pH-modifiers was carried out under accelerated conditions (40 °C/75% RH and 60 °C) for 2 weeks. The appearance of DPG/TA, DPG/CA and DPG/MLI significantly changed due to deliquescence, and DP purity values of DPG/TA, DPG/CA and DPG/MLI stored at 40 °C/75% RH for 2 weeks were determined to be 47.7%, 38.0% and 21.9%, respectively. In contrast, the other formulations were stable without any morphological transition or chemical degradation. Photostability tests were conducted with chemically stable formulations of DPG and DPG with six acidic pH-modifiers. These formulations were exposed to simulated sunlight consisting of UVA/B and visible light (250 W/m^2) for 24 h (Table 1). DPG and DPG with acidic pH-modifiers were stable after light exposure without any photodegradation. On the basis of the stability data under accelerated conditions and light exposure, the addition of some acidic pH-modifiers, such as TA, CA and MLI, had an impact on the stability of DP, possibly due to high hygroscopicity. The other six acids had no significant influence on DP stability, and further assessments were carried out to select a potential acidic pH-modifier.

3.2. Dissolution behavior of dipyridamole granule with pH-modifier

To clarify possible improvement of the dissolution behavior of DPG and DPG with acid, dissolution tests in 0.05 M phosphate buffer (pH 6.8) were carried out (Table 1). The DPG exhibited a limited initial dissolution rate of 4.2×10^{-2} h^{-1} and maximum level of drug dissolution reached 20% of total drug amount because of poor DP solubility at pH 6.8 (ca. 6 μg/mL at 37 °C) (Heigoldt et al., 2010). The dissolution profiles of DPG/SA, DPG/AA, DPG/D and DPG/E were almost the same as that of DPG, suggesting poor pH-modulating function of these four acids. In contrast, DPG/TA, DPG/CA, DPG/MLI, DPG/FA and DPG/TS exhibited higher initial dissolution rates by at least 10-fold and a maximum level of drug dissolution at pH 6.8 by at least 3-fold compared with DPG. To clarify enhancement of DP dissolution, C/Cs values were calculated from the actual DP concentration in dissolution medium (C) and DP solubility (Cs) (Fig. 2A). The C/Cs values of DPG/TA, DPG/CA, DPG/MLI, DPG/TS and DPG/FA were found to be ca. 4.1, 3.9, 3.1, 3.1 and 2.8, respectively. However, addition of the other acids failed to improve DP dissolution behavior.

Microenvironmental pH could vary depending on the type of acid, so different modulation of DP dissolution behavior was observed. The pH values of the acid-saturated solutions of TA, CA,

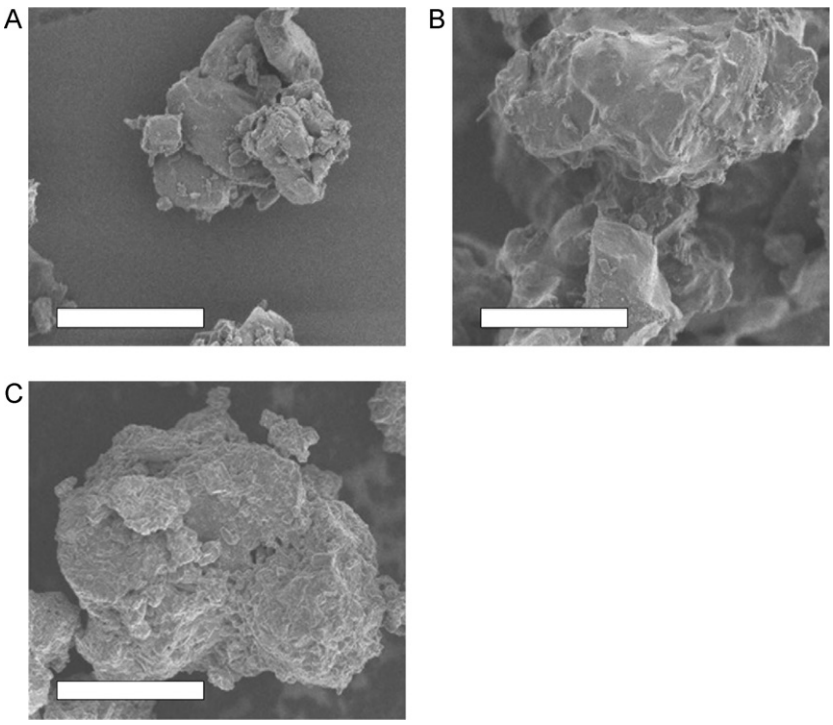

Fig. 1. Morphological observation of dipyridamole granule using scanning electron microscopy: (A) DPG, (B) DPG/TA and (C) DPG/TS. Each bar represents 25 μm.

MLI, TS and FA were 2 or lower, and those of the other acids, such as SA, AA, D and E, were above 2 (2.5–3.7). Considering the solubility of DP drug substance in various pH media (Heigoldt et al., 2010), it would seem that modulation of microenvironmental pH to below pH 4 would be sufficient to improve DP dissolution behavior. However, this was not the case. Thus, in addition to the solubility of DP, IDR was also considered in order to verify the reason for obtaining different dissolution behaviors depending on the acids used in the present study. IDR was defined as the dissolution rate of drug substance (Issa and Ferraz, 2011), and IDR showed a correlation with the modulation of microenvironmental pH. IDR values of DP at pH 2, pH 3 and pH 4 were 2.7 mg/cm^2/min, 0.62 mg/cm^2/min and 0.05 mg/cm^2/min, respectively, and changed significantly from pH 2 to pH 4 (Fig. 2B). In a previous study, Yu et al. (2004) suggested that IDR values below 0.1 mg/cm^2/min could be assigned as low soluble, and Zakeri-Milani et al. (2009) suggested IDR values less than 1 mg/cm^2/min could be assigned as low soluble. According to the present results, taken together with previous observations, DP at above pH 3 was considered to be poorly soluble. On the basis of the solubility and IDR values of DP, changing of the microenvironmental pH might have a big impact on dissolving DP in the range of pH 2–4, and modulation of microenvironmental pH to below 3 might be necessary to improve DP dissolution behavior. These five acids, TA, CA, MLI, TS and FA, seemed to provide sufficient microenvironmental pH, resulting in high dissolution behavior of DP from acidic pH to neutral pH.

On the basis of the manufacturability, stability and dissolution behavior, DPG/FA and DPG/TS could be available as DP formulations with acidic pH-modifiers. By comparison of these two formulations, maximum DP dissolution rate of DPG/TS was 1.2-fold higher than that of DPG/FA. TS is a white solid and an organic acid with pK_a value of −1.34, and its melting point is 106–107 °C (Stahl and Wermuth, 2011). According to FDA database, the acceptable daily intake (ADI) of TS is 7 mg/kg/day, and TS has been widely used as counterion for forming salts of basic or zwitterionic drugs for oral use, including suplatast and clofilium. Therefore, TS might be a promising pH-modifier for solubilizing DP, possibly leading to improved pharmacokinetic behavior of DP in a hypochlorhydric condition.

Table 1
The results on preparation and stability study of dipyridamole granule.

DP granule with acid (DPG/acid)	Stability test/purity (%)				Dissolution test at pH 6.8	
	Initial	40 °C/75% RH, 2 W	60 °C, 2 W	Photostability[a]	Initial dissolution rate (h^{-1})	Max drug dissolved level (% of total)
DPG	96.9	96.5	96.3	96.7	4.2×10^{-2}	20
DPG/TA	96.5	47.7[b]	5.7[b]	Not analyzed	7.3×10^{-1}	89
DPG/CA	94.9	38.0[b]	9.5[b]	Not analyzed	6.8×10^{-1}	85
DPG/MLI	82.1	21.9[b]	6.4[b]	Not analyzed	5.5×10^{-1}	69
DPG/TS	101.4	99.6	95.9	99.4	5.0×10^{-1}	69
DPG/SA	97.2	96.4	95.7	96.0	6.7×10^{-2}	20
DPG/AA	99.0	98.4	98.1	100.8	5.0×10^{-2}	19
DPG/FA	99.5	98.1	97.9	98.3	4.5×10^{-1}	60
DPG/D	98.9	96.7	98.1	96.3	3.3×10^{-2}	20
DPG/E	98.0	96.3	98.5	97.8	3.3×10^{-2}	20

[a] UV and visible light irradiation (250 W/m^2 for 24 h).
[b] Granule deliquesced during storage.

Table 2
The results on preparation and stability study of dipyridamole granule with *p*-toluenesulfonic acid.

DP granules with TS (TS ratio %)	Appearance of the granule		Stability/purity (%)			Photostability[a]/purity (%)
	After granulation	After drying and milling	Initial	40 °C/75% RH, 4 W	60 °C, 4 W	
DPG	Wet granule	Dried granule	98.3	98.1	97.7	99.7
DPG/TS15	Wet granule	Dried granule	100.5	101.8	99.9	100.4
DPG/TS22.5	Wet granule	Dried granule	98.3	96.4	99.1	97.2
DPG/TS30	Partly candy-like	Dried granule	101.5	95.2	99.7	101.4
DPG/TS45	Candy-like	Dried granule with lump	103.8	86.7[b]	102.0	101.9
DPG/TS60	Candy-like	Dried granule with lump	101.8	81.1[b]	96.6[b]	100.9

[a] UV and visible light irradiation (250 W/m^2 for 24 h).
[b] Granule deliquesced during storage.

3.3. Optimization of p-toluenesulfonic acid amount for dipyridamole granule

To optimize DPG/TS, DPG with various contents of TS, ranging from 15% to 60%, were prepared (Table 2). DPG with 15% loading of TS (DPG/TS15) and DPG/TS22.5 did not result in any problems in preparation. The DPG/TS30 had a partly candy-like appearance after wet granulation; however, fine granule was obtained after the drying process. In contrast, the appearance of DPG/TS45 and DPG/TS60 was found to be granular with lumps even after drying. These observations show that the manufacturability of DP granule worsens as the amount of TS increases. In addition, DPG/TS45 and DPG/TS60 were hygroscopic and unstable under conditions of 40 °C/75% RH for 4 weeks as evidenced by the decrease in purity to ca. 80%. The physical states of DPG and DPG/TS were evaluated by a powder X-ray diffraction (PXRD) (data not shown), in which DPG and DPG/TS exhibited typical diffraction patterns for the used excipients and crystalline DP. There is no denying that DP and TS might react during manufacturing process to partially form in situ salt, although these observations suggested that manufacturing processes would not induce severe polymorphic transformation, hydration and/or amorphization.

As shown in Fig. 3, dissolution profiles of all DPG formulations at 0.1 M HCl solution (pH 1.0) were almost identical, and the dissolved rate of DP reached ca. 100% of the total drug amount within 15 min because of high DP solubility at pH 1.0 (53.0 mg/mL at 37 °C) (Heigoldt et al., 2010). In contrast, the dissolution behavior of DPG at pH 6.8 was highly limited compared with that at pH 1.0 owing to DP pH-dependent solubility. Increasing contents of TS as the pH-modifier improved the dissolution behavior of DPG at pH 6.8, and maximum levels of the drug dissolved rate of DPG/TS15, DPG/TS30 and DPG/TS60 were higher than that of DPG (ca. 9%) by 2-, 4- and

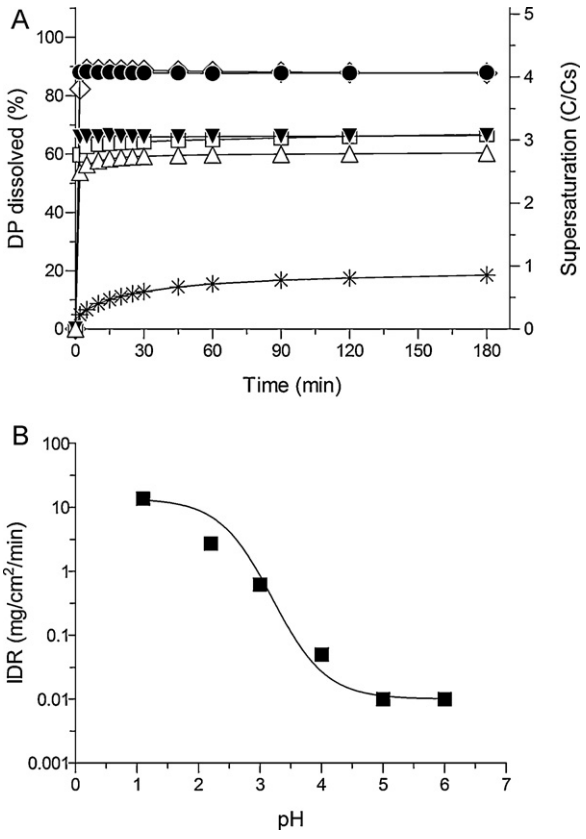

Fig. 2. Dissolution characterization of dipyridamole: (A) dissolution profiles of dipyridamole granule formulations at pH 6.8. (✳) DPG; (●) DPG/TA; (◊) DPG/CA; (▼) DPG/MLI; (□) DPG/TS; and (△) DPG/FA. Degrees of supersaturation are expressed as measured concentration of dissolved DP (C) vs. equilibrium solubility of DP (Cs). Each bar represents mean ± SD of 3 independent experiments. (B) Intrinsic dissolution rate of DP.

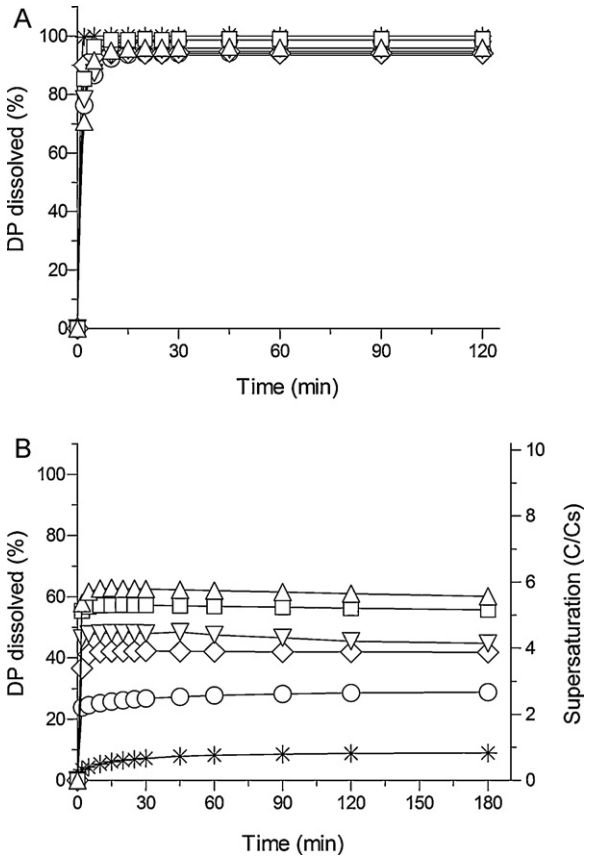

Fig. 3. Dissolution profiles of dipyridamole granule formulations at (A) pH 1.0 and (B) pH 6.8. (✳) DPG; (○) DPG/TS15; (◊) DPG/TS22.5; (▽) DPG/TS30; (□) DPG/TS45; and (△) DPG/TS60. Degrees of supersaturation are expressed as measured concentration of dissolved DP (C) vs. equilibrium solubility of DP (Cs). Each bar represents mean ± SD of 3 independent experiments.

Table 3
Pharmacokinetic parameters of dipyridamole granules following oral administration in normal and omeprazole-treated rats.

	API (3 mg/kg; i.v.)	DPG (10 mg/kg; p.o.)		DPG/TS30 (10 mg/kg; p.o.)	
		Normal rats	Omeprazole-treated rats	Normal rats	Omeprazole-treated rats
C_{max} (ng/mL)	716.4 ± 72.1	145.3 ± 19.9	68.8 ± 9.9	145.2 ± 15.5	148.2 ± 3.3
T_{max} (h)	0	0.25	0.25	0.38 ± 0.07	0.44 ± 0.06
$T_{1/2}$ (h)	0.24 ± 0.02	2.46 ± 0.49	3.54 ± 0.98	3.36 ± 0.68	2.75 ± 0.13
AUC (ng h/mL)	434.7 ± 96.9	395.3 ± 15.3	313.7 ± 63.1	476.2 ± 68.9	505.6 ± 4.7
AUC_{0-3} (ng h/mL)	384.2 ± 97.9	209.7 ± 29.9	130.3 ± 23.5	269.8 ± 23.8	237.5 ± 8.7
Oral BA (%)	–	27.3	21.7	32.9	34.8
Oral BA_{0-3} (%)	–	16.4	10.2	21.1	18.5

C_{max}: maximum concentration; $T_{1/2}$: half-life; and AUC: area under the curve of plasma concentration; BA: bioavailability. Values are expressed as means ± SE from 4 experiments.

6-fold, respectively (Fig. 3B). To clarify the supersaturation level of DP dissolution behavior, the C/Cs values were calculated. The C/Cs values of DPG/TS15, DPG/TS30 and DPG/TS60 were found to be ca. 2.5, 3.3 and 5.5, respectively (Fig. 3B), and supersaturated DP concentration in all DPG/TS formulations remained after 12 h (data not shown). Thus, the DP formulation with higher TS loading led to better dissolution behavior than that with lower TS loading. The results in this study are similar to those of a previous study using FA (Siepe et al., 2008). The reason for this was considered to be that DP formulation with high TS loading could control low microenvironmental pH more than that with low TS loading. On the basis of the results of manufacturability, stability and dissolution behavior, DPG/TS30 might be an appropriate DP formulation.

3.4. Pharmacokinetic profiling of dipyridamole granule

DP showed low oral bioavailability in hypochlorhydric patients with increased gastric pH because of DP pH-dependent solubility (Russell et al., 1994). The observation on the improved dissolution properties of DP formulation prompted us to clarify the possible improvement in the oral bioavailability of DP, and therefore the pharmacokinetic study with DPG and DPG with TS was performed using omeprazole-treated rats as a hypochlorhydric model. The rats were treated with 30 mg/kg omeprazole by oral administration, and then their gastric pH temporarily increased to above 6 within 1 h and maintained this level for about 5 h (Wada et al., 2006). Pharmacokinetic behavior of DP was evaluated after oral administration of DPG and DPG/TS30 (10 mg DP/kg) in normal rats in order to compare it with the pharmacokinetic behavior of DP in omeprazole-treated rats. Fig. 4 shows the plasma concentration–time profiles of DP in rats after oral administration, and relevant pharmacokinetic parameters including maximum concentration (C_{max}), time to maximum concentration (T_{max}), half-life ($T_{1/2}$) and AUC are given in Table 3. To clarify early systemic exposure after oral administration of DP formulation, AUC_{0-3} values for DP were also calculated. The oral administration of DPG (10 mg DP/kg) and DPG/TS30 (10 mg DP/kg) produced a pharmacokinetic behavior similar to that of DP. The plasma DP concentration for both formulations elevated rapidly after administration. The C_{max} values of DPG and DPG/TS30 formulations were 145.3 ± 19.9 ng/mL and 145.2 ± 15.5 ng/mL, respectively, and the AUC_{0-3} values of the two formulations were calculated to be 209.7 ± 29.9 ng h/mL and 269.8 ± 23.8 ng h/mL, respectively. On the basis of the AUC_{0-inf} value of intravenously administered DP (3.0 mg DP/kg), absolute bioavailabilities of DPG and DPG/TS30 in the normal rats were calculated to be ca. 27% and 33%, respectively. This result was expected because gastric pH in normal rats could be low and both formulations exhibited similar rapid dissolution behaviors at pH 1 (Fig. 3A).

In contrast, pharmacokinetic behavior after oral administration of DPG in omeprazole-treated rats (30 mg/kg) was found to be much lower than that in normal rats, and the C_{max} and AUC_{0-3} values were 68.8 ± 9.9 ng/mL and 130.3 ± 23.5 ng h/mL, respectively. These C_{max} and AUC_{0-3} values for oral administration of DPG in the omeprazole-treated rats decreased by ca. 53% and 38%, respectively, compared with those in normal rats. On the other hand, the pharmacokinetic behavior after oral administration of DPG/TS30 in the omeprazole-treated rats did not significantly change compared with that in the normal rats. The

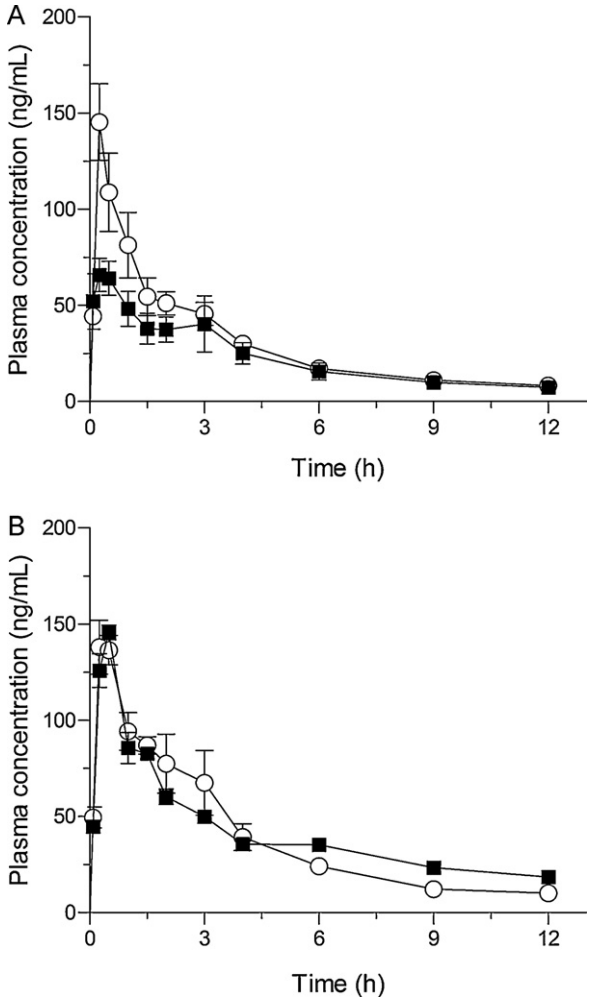

Fig. 4. Plasma dipyridamole concentrations in normal and hypochlorhydric rats after oral administration of dipyridamole granule: (A) DPG (p.o., 10 mg DP/kg) and (B) DPG/TS30 (p.o., 10 mg DP/kg). (○) Normal rats; and (■) hypochlorhydric rats. Data represent mean ± SE of 4 experiments.

C_{max} and AUC_{0-3} values of DPG/TS30 in the omeprazole-treated rats were 148.2 ± 3.3 ng/mL and 237.5 ± 8.7 ng h/mL, respectively, and did not change significantly in comparison with those in normal rats. These pharmacokinetic results corresponded well with the dissolution results at pH 6.8, demonstrating a high dissolution profile of DPG/TS30. TS as acidic pH-modifier in DP formulation improved pH-dependent dissolution behavior, and improvement of dissolution behavior led to improved DP oral bioavailability in hypochlorhydric patients with high gastric pH.

4. Conclusion

In this study, DP granules were prepared using ten acidic pH-modifiers with conventional wet granulation, and their physicochemical and pharmacokinetic properties were characterized. On the basis of these results, TS was selected as the appropriate acidic pH-modifier for DP formulation to achieve pH-independent dissolution, and the TS amount was optimized as 30% loading in DPG from the viewpoint of chemical stability and manufacturability. In the pharmacokinetic study using omeprazole-treated rats as a hypochlorhydric model, DPG exhibited significantly poor systemic exposure, although marked improvement in pharmacokinetic behavior of DP was observed after oral administration of the DPG/TS. DPG/TS with pH-independent dissolution behavior would allow high absorption of DP even under conditions with high gastric pH, possibly leading to enhanced therapeutic potential of DP in hypochlorhydric patients.

Acknowledgements

We wish to thank Dr. Peter Sieger, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, for providing IDR data of DP, and Dr. Ichiro Tsunenari, Nippon Boehringer Ingelheim Co., Ltd., Kobe, Japan, for providing the safety information of TS. This work was supported in part by a Grant-in-Aid for Young Scientists (B) (no. 22790043; S. Onoue) from the Ministry of Education, Culture, Sports, Science and Technology.

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