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Improved dissolution and pharmacokinetic behavior of dipyridamole formulation with microenvironmental pH-modifier under hypochlorhydria

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

[a] Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, 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

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ABSTRACT

The present study aimed to develop and characterize new formulations of dipyridamole (DP), a pH-dependent poorly soluble drug, employing an acidic pH-modifier for improving dissolution and absorption under hypochlorhydric condition. Granule formulations of DP (DPG) with and without fumaric acid (FA) were prepared with wet granulation, physicochemical properties of which were characterized focusing on morphology, dissolution and stability. Pharmacokinetic profiling of orally dosed DPG or DPG with 60% loading of FA (DPG/FA60) was carried out in omeprazole-treated rats as a hypochlorhydric model. Although pH-dependent dissolution behavior was observed in DPG, DPG/FA exhibited high rate and extent of dissolution in both acidic and neutral media. Complete supersaturation was achieved with a 2 h testing period in pH6.8 medium, and co-existing fumaric acid had no impact on the chemical/photochemical stability of DP in solid-state. After oral administration of DPG or DPG/FA60 (10 mg-DP/kg), there was ca. 40% reduction of AUC_{0-3} for DPG in omeprazole-treated rats as compared to that in normal rats; however, AUC_{0-3} for DPG/FA60 under hypochlorhydria was almost identical to that of DPG in normal rats. Given the improved systemic exposure early after oral administration in hypochlorhydric rats, the DPG/FA might provide better clinical outcomes in hypochlorhydric patients.

1. Introduction

Dipyridamole [2,6-bis(diethanolamino)-4, 8-dipiperidinopyrimido[5,4-*d*]pyrimidine] (DP), a phosphodiesterase inhibitor, has been clinically used for prevention and treatment of post-operative thromboembolic complications and cerebrovascular diseases (Kim and Liao, 2008; Zhou et al., 2005). Recent clinical studies have suggested the beneficial properties of DP for vasculature such as inhibition of proliferation, antioxidant and anti-inflammatory properties (Kim and Liao, 2008), thereby initiating its reevaluation as a vascular protecting agent (Schaper, 2005). In spite of its therapeutic potential, the oral dosage form of DP exhibits variable absorption with limited oral bioavailability of DP ranging from 11% to 44% (Terhaag et al., 1986). Since DP is a weak base with a pK_a value of 6.4 (Zhou et al., 2005), its water solubility is strongly dependent on the pH value of digestive fluids as DP dissolves readily in the stomach but poorly in the intestine (Patel and Patel, 2007). Hence, sufficient gastric acidity is believed to be an indispensable prerequisite for adequate dissolution and stable absorption of DP in vivo (Derendorf et al., 2005).

Low secretion of hydrochloric acid often occurs in young infants, ca. 30% of elderly (Champagne, 1989), subjects infected with *Helicobacter pylori* (Lahner et al., 2009) or human immunodeficiency virus (HIV) (Shelton et al., 1997), and patients treated with anti-ulcer drugs. Under these hypochlorhydric conditions, high variations in gastric acidity could result in inter- and intra-subject variability in the dissolution and absorption profiles of DP, possibly leading to the inconsistent clinical outcomes. Previous studies in healthy elderly patients also demonstrated that increasing gastric pH with H_2 receptor antagonist dramatically decreased DP absorption (Derendorf et al., 2005). To improve the dissolution behavior of

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve of blood concentration vs. time; BA, bioavailability; C_{max}, maximum concentration; CV, coefficient of variation; DP, dipyridamole; DPG, granule formulation of dipyridamole; FA, fumaric acid; HCl, hydrochloric acid; HPC, hydroxypropyl cellulose; PXRD, powder X-ray diffraction; RH, relative humidity; SEM, scanning electron microscopy; SIR, selected ion recording; $T_{0.5}$, half-life; T_{max}, time to maximum concentration; UPLC/ESI-MS, ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry; UV, ultraviolet.

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

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DP, several approaches have been used, including a self-emulsifying drug delivery system (Guo et al., 2011), cyclodextrin formulation (Rao et al., 2011), solid dispersion (Chen et al., 2007), and microparticles (Nilkumhang et al., 2009). Another effective methodology for manipulating the release of DP is to modify the microenvironmental pH around the DP particles/molecules (Siepe et al., 2006a,b). Thus, a number of efforts have been made to enhance and control the release of DP; however, far less is known about the pharmacokinetic behavior of the modified-release formulation of DP under hypochlorhydric condition.

The acidic pH-modifiers are commonly used to manipulate the pH in order to enhance the solubility of the basic drugs in the microenvironment of the dissolving dosage form. The increase in solubility could eliminate the supersaturation in the microenvironment or decrease the degree of supersaturation, leading to prevention or delay of the drug crystallization in the microenvironment. Thus, the pH-modifier approach might provide the opportunity for the drug to diffuse to the bulk medium and escape precipitation long enough to allow for absorption (Badawy and Hussain, 2007). The present study was undertaken to develop a granule formulation of DP (DPG) with an acidic pH-modifier for improving dissolution properties and oral bioavailability of DP with low variation under hypochlorhydric condition. In the present study, the DPGs were prepared with and without 15–60% (w/w) loading of pH-modifying fumaric acid, physicochemical properties of which were characterized with a focus on morphology by scanning electron microscopy (SEM), crystallinity by powder X-ray diffraction (PXRD), dissolution in acidic and neutral conditions, and chemical/photochemical stabilities. Pharmacokinetic profiling of DP after the oral administration of DPGs in both normal and omeprazole-treated rats as a hypochlorhydric model was conducted using ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry (UPLC/ESI-MS).

2. Materials and methods

2.1. Chemicals

Dipyridamole (DP) was manufactured 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). Fumaric acid was purchased from Bartek Ingredients Inc. (Ontario, Canada). Hydrochloric acid (HCl), ammonium formate, and ammonium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol and acetonitrile (liquid chromatography grades) were purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were purchased from commercial sources.

2.2. Granule formulation of dipyridamole with pH-modifier

2.2.1. Preparation

The DPG and DPG with fumaric acid (DPG/FA) were prepared to have a 30% (w/w) loading of DP and 0–60% (w/w) loading of fumaric acid with the remainder consisting of mannitol and HPC. Briefly, for preparation of DPG/FA15, DPG/FA30 or DPG/FA60, DP (30%), mannitol and fumaric acid (15%, 30% or 60%) were gently mixed, followed by addition of 5% HPC solution into the mixture, and they were granulated with mortar and pestle. The granules were dried at 60 °C by the vacuum drying oven, DP23 (Yamato Scientific Co. Ltd., Tokyo, Japan) for 2 h, and the dried granules were sieved through a 1 mm-mesh screen.

2.2.2. Dipyridamole determination

The amount of DP in the obtained granules was determined by the HPLC system with UV detection at 410 nm, Waters Alliance 2695 with Dual absorbance detector 2487 (Waters Corporation, Milford, MA). An Inertsil ODS-2 column (particle size: 5 μm, column size: 3.0 mm × 60 mm; GL Sciences Inc., Torrance, CA) was used and column temperature was maintained at 40 °C. Samples were separated using isocratic mobile phase consisting of 0.48 M ammonium formate (pH6.5), methanol and acetonitrile (29:12:9) with a flow rate of 1.0 mL/min, and retention time of DP was ca. 15 min.

2.3. Scanning electron microscopy (SEM)

Representative SEM images of DPG or DPG/FA 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.

2.4. Powder X-ray diffraction (PXRD)

PXRD pattern was collected using Mini Flex II (Rigaku Corporation, Tokyo, Japan) with Cu Kα radiation generated at 15 mA and 30 kV. Data were obtained from 3° to 33° (2θ) at a step size of 0.1° and scanning speed of 4°/min.

2.5. Stability testing

2.5.1. Stress stability testing

Stability studies on the DPG and DPG/FA were carried out at 40 ± 2 °C/75 ± 5% relative humidity (RH) in a stability chamber SRH-15VEVJ2 (Nagano Science Co. Ltd., Osaka, Japan) or 60 ± 2 °C for 4 weeks in LH21-15 M (Nagano Science Co. Ltd.). Samples after storage were subjected to the HPLC analysis as described in Section 2.2.2 and color measurement using computer-controlled colorimeter, SQ2000 model (Nippon denshoku, Tokyo, Japan).

2.5.2. Photostability testing

For the photostability test, each granule containing 50 mg DP was weighed exactly and spread in a 25 mL clear glass bottle. Each sample was stored in the Atlas Suntest XLS+ (Atlas Material Technology LLC, Chicago, IL), equipped with a xenon arc lamp. Each sample was irradiated with a UVA/B and Vis light (300–800 nm, 250 W/m^2) for 24 h, and the remaining amount of DP in the granule was determined by HPLC as described in Section 2.2.2.

2.6. Dissolution testing

Dissolution tests were carried out for 120 min under paddle method at 50 rpm in 900 mL of 0.05 M phosphate buffer (pH6.8) and/or 0.1 M HCl solution (pH1) using the dissolution tester system with the UV automatic flow system, NTR-6100 (Toyama Sangyo Co. Ltd., Osaka, Japan) at 37 °C. Granules were weighed to keep the total amount of DP in the dissolution vessel constant at 50 mg. Samples were collected at the indicated periods and analyzed with the automatic UV flow cell at 298 nm for pH6.8 buffer solution and at 283 nm for 0.1 M HCl solution.

2.7. Pharmacokinetic studies

2.7.1. 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%). Animals were fasted

for 12 h before intravenous or oral administration of DP samples, and pharmacokinetic study was carried out in a parallel design. In the present study, rats were pretreated with 1 mL of omeprazole suspension (p.o., 30 mg/kg) to develop an experimental hypochlorhydric model for pharmacokinetic study as reported previously (Wada et al., 2006). 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.

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

Blood samples (200 μL) were collected from the tail vein at the indicated periods (5, 15 and 30 min, 1, 1.5, 2, 3, 4, 6, 9 and 12 h) after intravenous administration of DP (3.0 mg/kg) dissolved in 0.2% tartaric acid (pH2.4) or oral administration of 0.5 mL of DPG suspension or DPG/FA60 suspension (10 mg DP/kg) in rats ($n = 4$ for each group). Suspension of DPG and DPG/FA60 was immediately administered just after preparation of suspension. Each blood sample (200 μL) was centrifuged at 10,000 × *g* to prepare plasma samples. Then, 150 μL of methanol was added to the 50 μL of plasma sample, and the solution was centrifuged at 3000 × *g* for 10 min. The supernatant was filtrated through the 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 retention times of 1.53.

2.8. 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 testing of dipyridamole formulations

Several organic acids have been employed as pH-modifiers to achieve pH-independent release of weakly basic drugs; e.g., fumaric, sorbic and adipic acids for verapamil (Streubel et al., 2000), citric acid for vinpocetine (Nie et al., 2004), and fumaric acid for ZK811752 (Kranz et al., 2005). Previous study also demonstrated that addition of fumaric acid into the hydrophilic matrix tablets of DP led to accelerated dissolution (Siepe et al., 2006b). In the present study, fumaric acid was employed as a microenvironmental pH-modifier to prepare DP-loaded granule formulations containing 15%, 30% and 60% of fumaric acid (DPG/FA15, DPG/FA30, and DPG/FA60) with conventional wet-granulation method. According to SEM images, the bulk DP was composed of large and smooth-surfaced rectangular crystals at the micron-scale, ranging up to ca. 50 μm in length (Fig. 1A-I). In contrast, more regularly shaped particles of the DPG and DPG/FA60 were observed in Fig. 1A-II and III. There seemed to be a marked increase in the surface area of the material and closer contact between the DP and fumaric acid

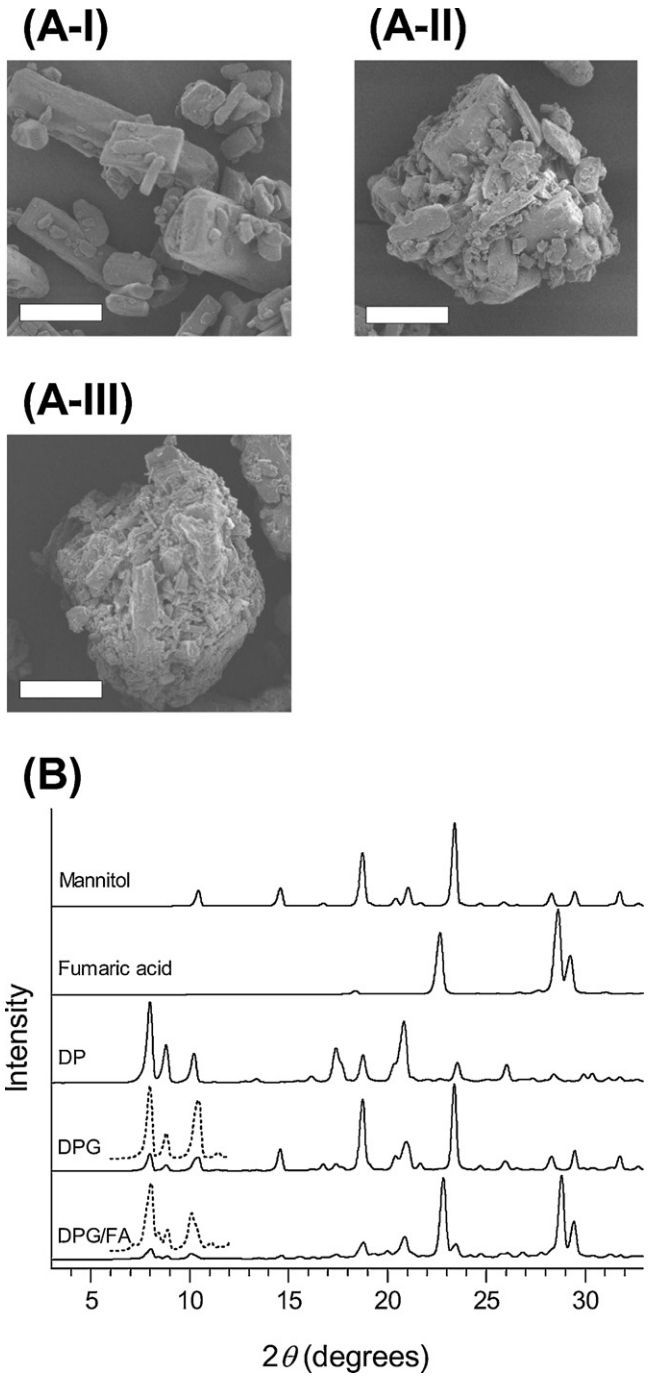

Fig. 1. Physicochemical characterization of dipyridamole-loaded granule formulations. (A) Scanning electron microscopic images from DP (I), DPG (II) and DPG/FA60 (III). Bar represents 20 μm. (B) Powder X-ray diffraction patterns of excipients, bulk DP, DPG, and DPG/FA60. Dotted lines represent magnification of partial diffraction patterns.

in the DPG/FA60. The physical state of DP formulations was further evaluated by PXRD (Fig. 1B). In the PXRD patterns of bulk DP, mannitol and fumaric acid, several characteristic peaks were observed, revealing their crystalline form. Both DPG and DPG/FA60 exhibited a diffraction pattern typical of the crystalline form of excipients, as well as small peaks for crystalline DP, although PXRD pattern on the DPG/FA60 indicated a very small but new peak at 8–9 degrees. These observations suggested that wet-granulation and subsequent drying processes might not induce severe polymorphic transformation, hydration and/or amorphization; however, there is

no denying that DP and FA would react during wet granulation to partially form in situ salt.

In general, the degradation rate of many pharmaceutical chemicals in solution is a function of solution pH, and the degradation rate and profile of those chemicals are also affected by the microenvironmental pH of the solid dosage form (Badawy and Hussain, 2007). To clarify the influence of co-existing fumaric acid on the chemical and physical stability of DP, stability testing for DPG and DPG/FA was carried out under accelerated conditions (Table 1). The DPG with high loading of fumaric acid, DPG/FA60 in particular, was expected to be somewhat degradable under accelerated conditions; however, this was not the case. According to the HPLC analysis of aged samples, no significant degradation was observed in either DPG or DPG/FA even after storage at 60 °C for 4 weeks. In addition, PXRD patterns on DPG and DPG/FA did not show any transition after storage at accelerated conditions. Thus, fine stability of the granulated product was confirmed despite its increased wettability. In addition, the DP molecule was found to be highly sensitive to UV light, resulting in photodegradation and color change under aerobic conditions (Vargas et al., 2001). In the present study, for photostability testing, DPG and DPG/FA were exposed to simulated sunlight consisting of UVA/B and visible light (250 W/m^2) for 24 h (Table 1). Light exposure to the DPG did not yield any photodegradants including 2-oxo-piperidinopyrimido compound, a major photoproduct (Vargas et al., 2001), and there was no color change. The DPG/FA also exhibited high photostability, whereas surface color of DPG/FA60 was slightly varied with a ΔE value of 5.7. The slight colorimetric transition would be indicative of the photoreactivity of DPG/FA60; however, results from HPLC analysis suggested that occurrence of photochemical reaction could be highly limited on the thin surface layer. PXRD analysis revealed high physical stability of DPG and DPG/FA under light exposure. On the basis of chemical and physical stability data under accelerated conditions and light exposure, addition of fumaric acid as pH-modifier into the DPG might not affect the stability of DP at least in the solid state. In some cases, microenvironmental pH-modifiers have no impact on the stability of active pharmaceutical ingredients, and moreover, modulation of microenvironmental pH sometimes provides an effective means for optimizing stability of certain solid systems as well as their dissolution behavior (Badawy and Hussain, 2007).

3.2. Dissolution behavior of dipyridamole formulations

To evaluate possible enhancement of dissolution behavior by microenvironmental pH-modifying approach, dissolution testing for DPG/FA was carried out in both HCl solution (pH1.0) and sodium phosphate buffer (pH6.8) to simulate normal and hypochlorhydric conditions, respectively. In the acidic condition (Fig. 2A), the DPG exhibited rapid drug releasing profile as dissolution of DP reached ca. 90% within 2 min. During the dissolution testing period (2 h), aggregation and precipitation of DP were negligible in the dissolution medium. Drug releasing characteristics for DPG/FA15 and DPG/FA30 were almost identical to that of DPG, and dissolution of DPG/FA60 in acidic medium reached 96% within 2 min and 100% after 15 min. These findings were consistent with previous observation of the rapid dissolution behavior of DP in acidic condition (Zhou et al., 2005), and modification of microenvironmental pH with high loading amount of fumaric acid might lead to a slight increase in the rate and extent of DP dissolution even at low pH.

In contrast to the acidic condition, poor dissolution behavior at pH6.8 was seen in the DPG at an initial dissolution rate of 0.19 ± 0.02 h^{-1} (Fig. 2B), since solubility of DP in pH6.8 medium

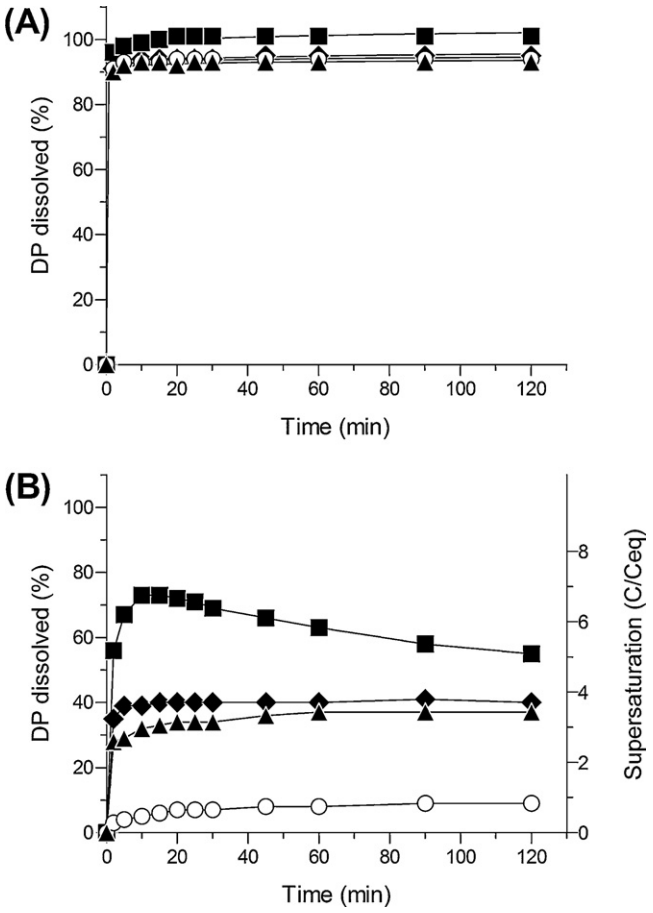

Fig. 2. Dissolution profiles of dipyridamole-loaded granule formulations differently treated in (A) acidic (pH1.0) and (B) neutral (pH6.8) media. ○, DPG; ▲, DPG/FA15; ♦, DPG/FA30; and ■, DPG/FA60. Degrees of supersaturation are expressed as measured concentration of dissolved DP (C) vs. equilibrium solubility of DP (Ceq). Each bar represents mean ± SE of 3 independent experiments.

Table 1
Stability data on granule formulations of dipyridamole.

	After 4 w-storage at accelerated conditions				After exposure to simulated sunlight[c]	
	40 °C/75% RH		60 °C		Assay (%)[a]	Color change (ΔE)[d]
	Assay (%)[a]	PXRD[b]	Assay (%)[a]	PXRD		
DPG	100.0	Not changed	99.6	Not changed	100.1	1.9
DPG/FA15	100.4	Not changed	101.1	Not changed	99.8	3.7
DPG/FA30	100.4	Not changed	101.4	Not changed	100.3	2.6
DPG/FA60	100.1	Not changed	99.7	Not changed	100.8	5.7

[a] % remaining to initial sample.
[b] Comparison with initial sample.
[c] Each sample was exposed to UVA/B and visible light (250 W/m^2) for 24 h.
[d] $\Delta E = \sqrt{((\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2)}$, where ΔL^*, Δa^*, and Δb^* represent the differences between the initial color and the specimen coordinates.

was found to be ca. 6 μg/mL (Heigoldt et al.). However, all the DPG/FA exhibited rapid dissolution behavior, and complete supersaturation could be achieved immediately after dispersion in water. In particular, there was marked improvement in dissolution from the DPG/FA60, the dissolution rates of which were calculated to be $14.9 \pm 2.8\ h^{-1}$. The DPG/FA60 also showed a significant increase in the extent of DP dissolution as evidenced by 6.8-fold greater concentration of the dissolved DP than equilibrium solubility (Ceq) of DP at 15 min. The supersaturated DP concentration in the DPG/FA60 gradually decreased to ca. 5-times Ceq at 2 h, whereas neither visible precipitation nor aggregation was seen during the experiment. DPG/FA15 and DPG/FA30 provided superior stability of the supersaturated DP solution with smaller supersaturation ratio of ca. 3.4 and 3.8, respectively, as compared to the DPG/FA60. In the presence of pH-modifier, the drug was thus capable of existing in a supersaturated state in the bulk solution for a relatively extended time period, whereas negligible change in pH value of dissolution media was observed during the dissolution testing. Based on these data, the pH-dependent dissolution of DP observed here was in agreement with previous reports (Diakidou et al., 2009), and the modification of microenvironmental pH with fumaric acid was effective for improving dissolution behavior of DP in neutral medium. The much higher supersaturated concentration and its duration at neutral pH have the potential to enhance the therapeutic potential of DP in hypochlorhydric patients, possibly due to improved dissolution and absorption in the gastrointestinal tract.

3.3. *Pharmacokinetic profiling of dipyridamole formulations under hypochlorhydria*

The absorption of DP is dissolution rate-limited over at least a part of the usual physiological range of gastrointestinal pH. Hypochlorhydric subjects commonly have high pH values in both the stomach and the intestine regardless of the prandial phase, so they exhibit a slow rate and poor extent of DP absorption (Derendorf et al., 2005). Preferable results from dissolution testing on the DPG/FA60 prompted us to clarify possible improvement in oral absorption of DP under hypochlorhydric condition. Previously, several animal models with reduced gastric acid were established that includes (i) a low gastric acidity model, (ii) a weak antacid model with an H_2 receptor antagonist, and (iii) a strong antacid model with magnesium hydroxide (He et al., 2004; Wada et al., 2006). In the present study, a weak antacid model was chosen as an experimental hypochlorhydric model for pharmacokinetic study, in which omeprazole (30 mg/kg) was co-administered to temporarily elevate gastric pH up to 6 (Wada et al., 2006).

First, for comparison purposes, pharmacokinetic behavior of DP was assessed after oral administration of DPG and DPG/FA60 (10 mg DP/kg) in normal rats. The plasma concentration–time profiles of DP in rats are shown in Fig. 3A, and relevant pharmacokinetic parameters including C_{max}, $T_{0.5}$, AUC_{0-inf}, and absolute bioavailability are listed in Table 2. In addition to these pharmacokinetic parameters, AUC_{0-3} values for DP were also calculated to compare

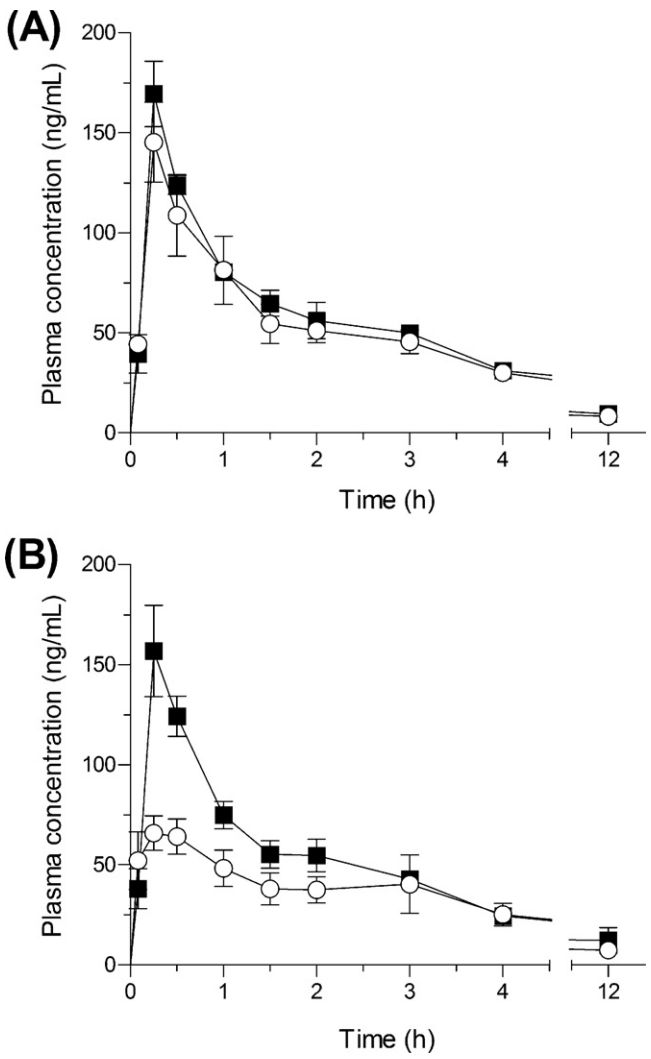

Fig. 3. Plasma concentration of dipyridamole in (A) normal and (B) hypochlorhydric rats after oral administration of granule formulations. ○, DPG (p.o., 10 mg DP/kg); and ■, DPG/FA60 (p.o., 10 mg DP/kg). Data represent mean ± SE of 4 experiments.

early systemic exposure after oral administration of DP formulations. Oral administration of the DPG resulted in rapid elevation of plasma DP levels up to C_{max} 145.3 ± 19.9 ng/mL, and the AUC_{0-inf} value was calculated to be 395.3 ± 15.3 ng h/mL. Similar pharmacokinetic behavior was seen for the DPG/FA60 with C_{max} and AUC_{0-inf} values of 169.4 ± 16.4 ng/mL and 414.5 ± 21.6 ng h/mL, respectively. On the basis of the AUC_{0-inf} value of intravenously administered DP (3.0 mg/kg), absolute bioavailabilities of DPG and DPG/FA60 in the normal rats were calculated to be ca. 27 and 29%, respectively. There was also no significant difference between each

Table 2
Pharmacokinetic parameters for granule formulations of dipyridamole following oral administration.

	C_{max} (ng/mL)	$T_{0.5}$ (h)	AUC_{0-3} (ng h/mL)[a]	AUC_{0-inf} (ng h/mL)	BA (%)
Normal rats					
DP (3 mg/kg, i.v.)	716.4 ± 72.1	0.24 ± 0.02	–	434.7 ± 96.9	–
DPG (10 mg DP/kg, p.o.)	145.3 ± 19.9	2.5 ± 0.5	209.7 ± 29.9 (28.5%)	395.3 ± 15.3	27.3
DPG/FA60 (10 mg DP/kg, p.o.)	169.4 ± 16.4	2.4 ± 0.4	224.6 ± 10.9 (9.8%)	414.5 ± 21.6	28.6
Rats treated with omeprazole (30 mg/kg)					
DPG (10 mg DP/kg, p.o.)	68.8 ± 9.9	3.5 ± 1.0	130.3 ± 23.5 (36.1%)	313.7 ± 63.1	21.7
DPG/FA60 (10 mg DP/kg, p.o.)	159.8 ± 20.4	2.3 ± 0.5	212.8 ± 15.3 (14.4%)	391.0 ± 36.9	27.0

[a] RSD values were indicated in parenthesis. C_{max}, maximum concentration; $T_{0.5}$, half-life; AUC_{0-3} or AUC_{0-inf}, area under the curve of blood concentration vs. time from $t = 0$ to $t = 3$ or ∞ after administration; and BA, bioavailability. Values are expressed as mean ± SE of 4 experiments.

of the AUC_{0-3} values, indicating a similar early systemic exposure of DP. These outcomes were not surprising considering the gastric acidity of normal rats and dissolution behavior of DP under acidic condition. However, in the rats treated with omeprazole (30 mg/kg), plasma DP level was found to be very low early after oral administration of the DPG, 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 (Fig. 3B). The plasma concentration of DP decreased gradually with apparent elimination kinetics of $0.18\ h^{-1}$. Thus, the orally administered DPG in the hypochlorhydric rats exhibited significant decrease in C_{max} and AUC_{0-3} values by ca. 53% and 38%, respectively, when compared to that in the normal rats. In contrast, the plasma concentration–time curve for DPG/FA60 in the hypochlorhydric rats was almost identical to that in the normal rats. These findings were relatively consistent with the results from the dissolution test in pH6.8 medium, demonstrating the accelerated dissolution behavior of DPG/FA60. There also appeared to be high inter-individual variation in AUC_{0-3} from the DPG in both normal and omeprazole-treated rats (coefficient of variation, CV: 29% in normal rats and 36% in hypochlorhydric rats). Interestingly, there was ca. 60–66% reduction of inter-individual variation in AUC_{0-3} from DPG/FA60 compared with that from the DPG. The individual variability in gastric acidity may affect the dissolution of DP in the gastric fluid and thereby cause inconsistent absorption. However, modification of microenvironmental pH led to pH-independent dissolution of DP even at neutral pH, and this might be attributable to improved and consistent absorption under gastric hypochlorhydria. From the pharmacokinetic behavior of the DPG/FA60, taken together with the pH-independent dissolution characteristics, the microenvironmental pH-modifying approach might provide better clinical outcomes from DP-based medication for treatment of thromboembolic complications and cerebrovascular disorders, especially, in patients with hypochlorhydria.

4. Conclusion

In the present study, granule formulation of DP was prepared by employing fumaric acid as a microenvironmental pH-modifier, allowing a pH-independent dissolution. Even in the presence of fumaric acid, no significant chemical or photochemical degradation of DP was seen in the DPG/FA under accelerated condition or UV irradiation. There appeared to be poor and inconsistent systemic exposure of DP early after oral administration of DPG in the hypochlorhydric rats; however, improved pharmacokinetic behavior of DP with low inter-individual variation was observed after oral administration of the DPG/FA. The pH-independent dissolution of DPG/FA might explain in part the high absorption under gastric hypochlorhydria. From these findings, the use of pH-modifier might be efficacious for increasing the therapeutic potential of DP in the hypochlorhydric patients.

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