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New Dipyridamole Salt with Improved Dissolution and Oral Bioavailability under Hypochlorhydric Conditions

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Summary: The aim of this study was to develop new dipyridamole (DP) salts with pH-independent solubility for improving oral bioavailability under hypochlorhydria. Salt screening was carried out using nine counterions by the temperature gradient method. Six DP salts were obtained, and there was marked improvement in dissolution behavior for all DP salts in a neutral medium. Most DP salts were stable under accelerated conditions. On the basis of the dissolution and stability data, DP tosylate (DP/TS) was selected as a promising DP salt. The pharmacokinetics of DP and the promising DP salt were assessed in normal rats and omeprazole-treated rats as a hypochlorhydric model. After oral administration of DP/TS (10 mg-DP/kg) in normal rats, enhanced DP exposures with increased C_{max} and AUC_{0-3} were observed compared with those with DP by *ca.* 2.8- and 1.7-fold, respectively. There was *ca.* 1 h delay of T_{max} and *ca.* 62% reduction of AUC_{0-3} for DP in omeprazole-treated rats compared with those for DP in normal rats; however, oral absorption for DP/TS under hypochlorhydria was almost identical to that in normal rats. The newly developed DP/TS might provide better therapeutic efficacy in clinical use for hypochlorhydric patients.

Keywords: dipyridamole; salt screening; dissolution; bioavailability; hypochlorhydria

Introduction

Dipyridamole [2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine] (DP) is a thromboxane synthase inhibitor that is clinically used as a vasodilatory agent and an antithrombotic agent.[1,2] DP is well known to be a weakly basic drug with a pK_a value of 6.4[3] and to exhibit pH-dependent solubility, with high solubility at acidic pH and low solubility above pH 4.[4] DP is categorized as a Biopharmaceutical Classification System (BCS) class II compound.[4] For BCS class II compounds with high permeability and low solubility, drug absorption is often limited by drug solubility and dissolution.[5] In clinical practice, a hypochlorhydric situation often occurs in young infants, *ca.* 30% of elderly,[6] subjects infected with *Helicobactor pylori*[7] or human immunodeficiency virus (HIV),[8] and patients treated with antiulcer drugs. In previous clinical research, oral absorption of DP was decreased in famotidine-treated patients with increased gastric pH, and the oral bioavailability of DP was reduced by *ca.* 60% with hypochlorhydric subjects compared with that in control subjects.[9] In addition to low secretion of hydrochloric acid, gastric pH could elevate after a meal,[9] and elevated gastric pH leads to incomplete oral absorption of DP with pH-dependent solubility, possibly leading to inconsistent clinical outcomes. Therefore, enhancement of DP solubility at neutral pH could improve oral bioavailability in hypochlorhydric patients.

Various techniques have already been developed to enhance solubility and dissolution behavior. In formulation development, solid dispersion,[10,11] micronization,[12,13] cyclodextrin complexation[14] and a microenvironmental pH-modifier approach[15–17] have already been performed to enhance the dissolution behavior of DP. In particular, the microenvironmental pH-modifier approach might be promising because of the simplified manufacturing process and cost performance. In our previous study, new DP immediate-release formulations were developed using the microenvironmental pH-modifier approach, and these DP formulations successfully improved dissolution behavior and oral absorption under hypo-

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chlorhydric conditions.[18,19] In the pharmaceutical industry, salt formation is a crucial step in drug development since it has a huge impact on physicochemical properties of drug substances.[20–22] In particular, salt formation has been identified as the most common and effective method of increasing solubility and dissolution rates of both acidic and basic drugs without modifying their chemical structures, and salt formation also provides a means of altering the hygroscopicity, stability, impurity profile and particle characteristics of a drug at reasonable cost. For DP, salt formation could also be a beneficial approach to improve dissolution and oral absorption. However, there are a limited number of reports and patents on salt formation of DP,[23] and their biopharmaceutical properties are far less well known.

The present study aimed to develop new DP salts with pH-independent solubility to improve oral bioavailability under hypochlorhydric conditions. DP salts were prepared using nine counterions by the temperature gradient method. The physicochemical properties of the obtained DP salts were characterized by polarized light microscopy (PLM), scanning electron microscopy (SEM), powder X-ray diffraction (PXRD) and thermogravimetric and differential thermal analyses (TG/DTA). Spectroscopic characterization of DP and DP salts was carried out using ultraviolet (UV) spectral analysis to anticipate a possible change of photosensitivity by salt formation. A promising salt was selected on the basis of the results on the physicochemical properties. A pharmacokinetic study on the promising DP salt was also performed to clarify the possible improvement in oral bioavailability using omeprazole-treated rats as a hypochlorhydric model.

Materials and Methods

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). 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). Ethyl acetate, tetrahydrofuran (THF), toluene, 2-propanol, hydrochloric acid (HCl), phosphoric acid (PA), sulfuric acid (SA), *p*-toluenesulfonic acid monohydrate (TS), benzenesulfonic acid monohydrate (BS) and maleic acid (MA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol and acetonitrile were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). All other chemicals were purchased from commercial sources.

Preparation of dipyridamole salts: Crystalline salts of dipyridamole were prepared using a 500-mg scale. Salt screening for DP was carried out in methanol, acetonitrile, 2-propanol, ethyl acetate, THF and toluene. The resultant products were filtrated and dried under a nitrogen atmosphere. The obtained solids were characterized by ion chromatography, inductively coupled plasma atomic emission spectrometry (ICP-AES), PXRD and TG/DTA.

Micrographic images:

Polarized light microscopy (PLM)

Representative PLM images of DP samples were taken using a BX53-P microscope (Olympus Co. Ltd., Tokyo, Japan). DP samples were examined under various conditions, including differential interference contrast, using slightly uncrossed polars and a red wave compensator.

Scanning electron microscopy (SEM)

Representative SEM images of DP samples 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.

Powder X-ray diffraction (PXRD) analysis: The PXRD pattern was collected with a D8 Advance (Bruker AXS GmbH, Karlsruhe, Germany) with Cu Kα radiation generated at 40 mA and 35 kV. Data were obtained from 3° to 35° (2θ) at a step size of 0.2° and scanning speed of 6°/min.

Thermal analysis: TG/DTA was performed using a Simultaneous DTA-TG Apparatus DTG-60 (Shimadzu Co., Ltd., Kyoto, Japan). TG/DTA thermograms were collected in an aluminum open-pan system using a sample weight of *ca.* 3 mg and a heating rate of 10°C/min with nitrogen purge at 20 mL/min.

Determination of water content in DP salts: The water content in DP salts was determined using a Karl Fischer Moisture Titrator, MKA-520 (Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan).

Ion chromatography: Ion chromatography was performed using an HIC-20A with a suppressor type of conductivity detector (Shimadzu Co., Ltd.), and Shim-pack IC-SA2 (Shimadzu Co., Ltd.) was used as a column for anion analysis. The solution of 0.6 mM sodium carbonate–12 mM sodium hydrogen carbonate was used as a mobile phase at a constant flow rate of 1.0 mL/min.

Inductively coupled plasma atomic emission spectrometry (ICP-AES): ICP-AES was performed using an ICP emission spectrometer ICPS-8100 (Shimadzu Co., Ltd.) at 180.731 nm for sulfur and 178.278 nm for phosphorus with argon gas as the carrier gas.

Moisture sorption analysis: Dynamic vapor sorption (DVS) was recorded using an Automated Water Sorption Analyser (DVS-1, Surface Measurement Systems Ltd., London, UK). For moisture sorption analysis, *ca.* 5 mg of each sample was stored under humidity levels that varied between 0 and 95% RH at 25°C.

Dipyridamole determination in salts: The amount of DP in the DP salt was determined using an HPLC system with UV detection at 410 nm, Waters Alliance 2695 with Dualλ absorbance detector 2487 (Waters Corporation, Milford, MA). An ODS column (particle size: 5 μm, column size: 3.0 mm × 60 mm; Inertsil ODS-2, GL Sciences, Inc., Torrance, CA) was used and column temperature was maintained at 40°C. Samples were separated using an isocratic mobile phase consisting 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.

Stability testing:

Stress stability testing

For stress stability study, *ca.* 30 mg of each DP salt was poured into a 25 mL brown 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.), for 12 weeks. After storage, the samples were evaluated to purity according to the section on Dipyridamole determination in salts.

Photostability testing

For photostability testing, each DP salt containing *ca.* 5 mg of DP was weighed exactly and spread in a 25 mL clear glass bottle. The samples were stored in a SUNTEST XLS+ (Atlas Material

Technology LLC, Chicago, IL) and the amount of DP remaining in the DP salt was determined by HPLC as described in the section on Dipyridamole determination in salts. The UVA/B and visible light irradiation (300–800 nm) was carried out at 25°C with an irradiance of 250 W/m^2 for 1 h and 24 h.

Dissolution properties:

Preliminary dissolution testing

Dissolution screening was carried out for 60 min at 200 rpm in 20 mL of 0.05 M monobasic potassium phosphate buffer solution (pH 6.8) using a small volume dissolution tester, the μ-DISS Profiler™ (Pion Inc., Woburn, MA) at 37°C. The DP salt was weighed to keep the total amount of DP in the dissolution vessel constant at *ca.* 3 mg. A sample volume of 5 mL was withdrawn at 60 min, filtrated through a 0.45-μm membrane filter and then measured with a UV-visible spectrophotometer UV-2550 (Shimadzu Co., Ltd.) at 298 nm.

Dissolution testing

Dissolution tests were carried out for 2 h by the paddle method at 50 rpm in 900 mL of 0.05 M monobasic potassium phosphate buffer solution (pH 6.8) and 0.1 M HCl solution (pH 1.0) using the dissolution tester system with a UV automatic flow system, NTR-6100 (Toyama Sangyo Co., Ltd., Osaka, Japan), at 37°C. The sample was weighed to keep the total amount of DP in the dissolution vessel constant at 50 mg. Samples were measured at the indicated times with an automatic UV flow cell at 298 nm for pH 6.8 buffer solution and at 283 nm for 0.1 M HCl solution. The supersaturation level was calculated from the actual DP concentration in the dissolution medium (C) and equilibrium DP solubility (C_{eq}) as the C/C_{eq} value.

UV spectral analysis: DP samples were dissolved in 50% methanol solution at a final concentration of 0.02 mg/mL. UV-visible absorption spectra were recorded with a UV-visible spectrophotometer, the UV-2550 (Shimadzu Co., Ltd.).

Diffuse reflectance UV-visible spectral analysis: The diffuse reflectance UV-visible spectra were measured using a UV-visible spectrophotometer, the UV-2550, equipped with an ISR-2200 integrating sphere attachment (Shimadzu Co., Ltd.). Barium sulfate was used as the standard, and the Kubelka-Monk function was applied to convert diffuse reflectance data to the absorbance spectra.

Pharmacokinetics:

Animals

Male Sprague–Dawley rats, weighing *ca.* 354 ± 11.6 g (10–11 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 RH (55 ± 5%). Animals were fasted for 18 h before oral administration of DP samples. In this 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.[24] 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.

UPLC/ESI-MS analysis for plasma concentration of dipyridamole

Blood samples (200 μL) were collected from the tail vein at the indicated periods (5, 15 and 30 min, and 1, 1.5, 2, 3, 4, 8 and 12 h) after oral administration of 0.5 mL of DP suspension or DP/TS suspension (10 mg DP/kg). A suspension of DP and DP/TS was immediately administered just after preparation of the suspension. Each blood sample (200 μL) was centrifuged at 10,000 × g for 5 min at 4°C to prepare plasma samples. To 50 μL of plasma sample, 150 μL of methanol was added, and the solution was centrifuged at 3,000 × 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), which included a 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 the 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 min.

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.

Results and Discussion

Preparation and physicochemical properties of dipyridamole salts: In the present study, salt screening of dipyridamole (DP) salts was conducted with nine counterions by the temperature gradient method, and these counterions were selected on the basis of oral toxicity and their pK_a values, as described in **Table 1**[25]; this was because a pK_a difference between acid and base (ΔpK_a) of at least 2 or 3 is generally required to form a salt.[26,27] Salt screening was firstly carried out in methanol, acetonitrile, 2-propanol, ethyl acetate, THF or toluene, and, on the basis of crystallization kinetics and yields, 2-propanol and THF were found to be suitable crystallization solvents (data not shown). The DP salts with six counterions were successfully obtained, and the yield of these salts was over 50% (**Table 1**); however, the other three acids, namely L-tartaric acid (TA), fumaric acid (FA) and citric acid (CA), did not form any DP salts. The differences in pK_a values between DP and each of these three counterions were below 3.5, and those for the other six counterions were above 4. This finding suggested that ΔpK_a values greater than 4 might be required for DP salt formation. Morphology of the DP and DP salts prepared in this study was assessed by SEM and PLM. Intense birefringence was observed in crystalline DP and the six DP salts in PLM appearance (**Fig. 1A**), and morphological difference was observed between DP and the DP salts in SEM images (**Fig. 1B**). SEM micrographs revealed a tablet-shaped morphology of DP, plate-like morphology of DP tosylate (DP/TS), irregular needle-like morphology of DP besylate (DP/BS) and rock-like morphology of DP hydrochloride (DP/HCl), DP phosphate (DP/PA), DP sulfate (DP/SA) and DP maleate (DP/MA). The physical state of the six DP salts obtained was evaluated by PXRD (**Fig. 2A**) and TG/DTA (**Fig. 2B**). DP and the six DP salts exhibited typical diffraction patterns, and significant differences in PXRD patterns among the samples were observed, suggesting that the six DP salts could be different crystal forms and could not remain as a free base of DP. On the basis of the results from TG/DTA, DP and all six DP salts showed thermal events, and the extrapolated onset temperature of the endothermic peak (T_{on}) for each sample was different. DP/HCl

Table 1. The results for preparation of dipyridamole salts

Counterion	MW[a]	pK_a[a]	ΔpK_a (DP–counterion)	Crystallization solvent	Stoichiometry[b]	Yield (%)
Hydrochloric acid (HCl)	36.46	−6	10.4	2-propanol	1:1 (H_2O)	62.7
Phosphoric acid (PA)	98.00	1.96, 7.12, 12.32	4.44	2-propanol	1:2	47.0
Sulfuric acid (SA)	98.08	−3, 1.92	10.4	2-propanol	1:2	58.6
p-Toluenesulfonic acid (TS)	172.20	−1.34	7.74	THF	1:2	93.7
Benzenesulfonic acid (BS)	158.17	0.7	5.70	2-propanol	1:2	48.6
L-Tartaric acid (TA)	150.09	3.02, 4.36	3.38	2-propanol	Not gained	Not gained
Fumaric acid (FA)	116.07	3.03, 4.38	3.37	2-propanol	Not gained	Not gained
Citric acid (CA)	192.12	3.13, 4.76, 6.40	3.27	2-propanol	Not gained	Not gained
Maleic acid (MA)	116.07	1.92, 6.23	4.48	2-propanol	1:1	91.3

[a]Data from Handbook of Pharmaceutical Salt (2002).[25]
[b]Stoichiometry was confirmed using ion chromatography, ICP-AES and KF.

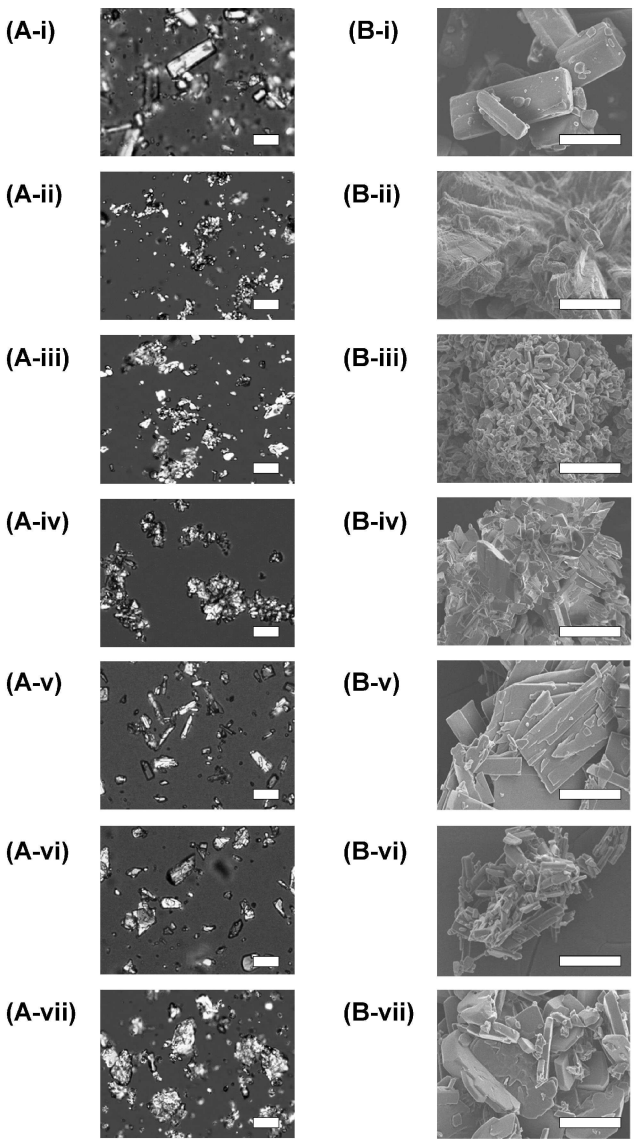

Fig. 1. Micrographic images of dipyridamole and dipyridamole salts
(A) Polarized light microscopy and (B) scanning electron microscopy observations. (i) DP, (ii) DP/HCl, (iii) DP/PA, (iv) DP/SA, (v) DP/TS, (vi) DP/BS and (vii) DP/MA. Each bar represents 20 µm.

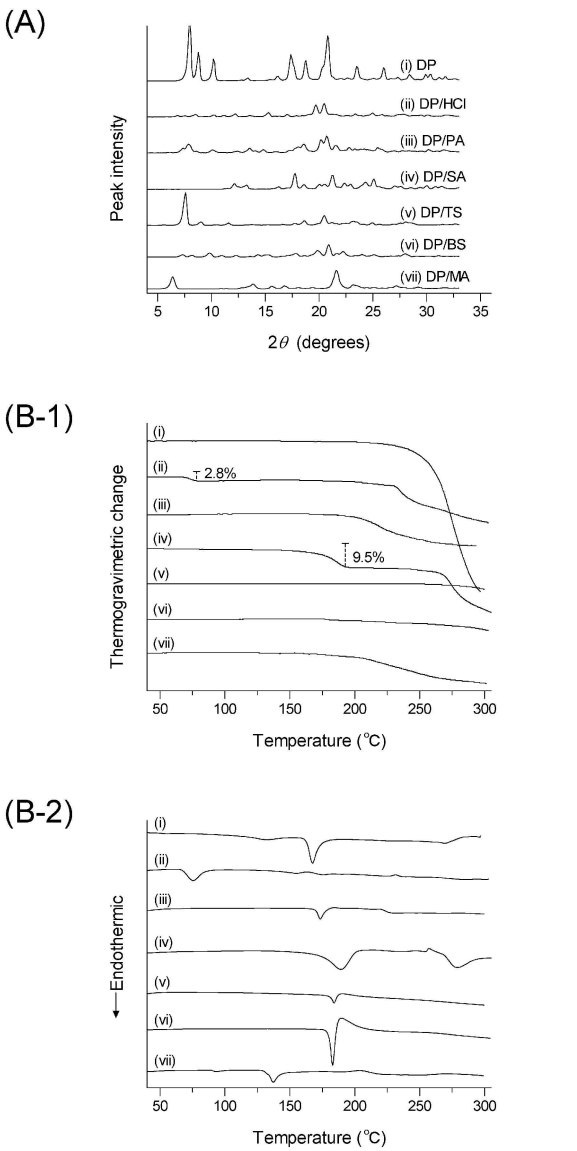

Fig. 2. Characterizations of dipyridamole and dipyridamole salts
(A) Powder X-ray diffraction patterns, (B-1) thermograms and (B-2) differential thermal analysis. (i) DP, (ii) DP/HCl, (iii) DP/PA, (iv) DP/SA, (v) DP/TS, (vi) DP/BS and (vii) DP/MA.

Table 2. The physicochemical and dissolution properties of dipyridamole salts

DP salt	Stability, 12 weeks % of DP remaining[a]		Photostability[b] % of DP remaining[a]		Dissolution at pH 6.8		DVS isotherm[c] change in mass (%)
	40°C/75% RH	60°C closed	1 h	24 h	DP dissolved at 60 min (%)	pH value of test medium after testing	25°C/95% RH
DP	98.8	98.9	99.6	98.5	10	6.78	0.051
DP/HCl	101.3	102.4	102.4	98.9	21	6.78	3.842
DP/PA	101.9	100.5	100.7	99.4	33	6.78	0.218
DP/SA	98.3	98.4	100.0	100.4	46	6.78	5.552
DP/TS	100.2	98.6	98.8	96.7	43	6.80	1.907
DP/BS	98.5	98.5	97.1	93.7	33	6.78	0.682
DP/MA	98.3	95.7	100.3	98.1	28	6.77	0.135

[a]Remaining to initial sample.
[b]UV and visible light irradiation (250 W/m^2).
[c]Sample was stored under humidity between 0 and 95% RH at 25°C.

and DP/SA exhibited endothermic weight loss, and the T_{on} of DP/HCl and DP/SA were at 62°C and 173°C, respectively. The results of PXRD and TG/DTA suggested that the obtained DP salts were different salt forms. The stoichiometry of the DP salts was assessed using ion chromatography and ICP-AES (data not shown). The molar ratio of counterion to DP in DP/PA, DP/SA, DP/TS and DP/BS was calculated to be approximately 2:1, and that in DP/MA and DP/HCl was approximately 1:1 (**Table 1**). The endothermic weight loss of DP/HCl in TG was *ca.* 2.8% from 60 to 80°C (**Fig. 2B-1**), and this finding, taken together with Karl-Fischer titration, was indicative of monohydrate.

Chemical and photochemical stability studies on dipyridamole salts: Stability testing of DP and the six DP salts was performed under accelerated conditions (40°C/75% RH and 60°C) for 12 weeks to clarify the influence of salt formation on the chemical stability (**Table 2**). The appearance of DP and DP salts did not change after storage. According to the HPLC measurement of aged samples, very small unidentified peaks were observed in DP salts (data not shown); however, most samples maintained the high potency (>98%) even after storage at accelerated conditions. After storage at 60°C for 12 weeks, only DP/MA exhibited slight degradation (*ca.* 4%), suggesting the potential influence of co-existing MA on the chemical stability of DP. In general, the hygroscopic property of a drug substance tends to impact upon its chemical and physicochemical stability; therefore, water sorption of DP and DP salts was evaluated. DP salts exhibited higher water sorption than DP. In particular, DP/SA and DP/HCl showed remarkably higher water sorption, suggesting that the stability of DP salts might be influenced by humidity conditions (**Table 2**). However, after storage at 40°C/75% RH for 12 weeks, the amount of remaining DP in all DP salts including DP/HCl and DP/SA did not change, showing that the stability of DP salts might not be influenced by moisture. The physical states of DP and DP/TS as representative samples were evaluated by PXRD and TG/DTA, and neither DP nor DP/TS showed any transition after storage under accelerated conditions for 12 weeks (data not shown). In a previous study, the photo-oxidation reaction of DP coexisting with hydrate silica was found to become higher than that of DP by itself.[28] Interaction between DP and counterions in salt formation has the potential to change the UV-absorbing property of DP, and a change in the UV-absorbing property might also influence photostability. Therefore, spectroscopic analyses of DP and DP salts were carried out using UV spectral analysis in solution and diffuse reflectance UV-visible solid-state absorption spectral analysis. DP/SA, DP/TS and DP/BS were selected as representative samples for this analysis because SA, TS and BS are strong acids and could interact with DP strongly compared with the other weak acids. UV spectral patterns in 50% methanol solution for all three DP salts were almost the same; however, their spectral patterns were different from that of DP. All samples showed strong absorption in visible light (400–700 nm), and the maximum absorption of DP was shifted from 412 nm to 405–406 nm by salt formation (**Fig. 3A**). Thus, the UV spectral patterns of DP salts were found to be different from that of DP, and this result was in agreement with the UV-visible diffuse reflectance spectra in a solid state (**Fig. 3B**). This finding suggested that the light sensitivity of DP salts could be different from that of DP; therefore, a photostability study was performed for the DP salts. DP and the DP salts were exposed to simulated sunlight consisting of UVA/B and visible light (250 W/m^2) for the indicated periods (**Table 2**). Decreases of the remaining DP were observed in DP/TS and DP/BS by *ca.* 3% and 6%, respectively; however, most DP salts showed no significant changes in the amount of remaining DP after light exposure compared with DP. Although the UV spectral pattern of DP salts shifted to that of DP, the DP salts exhibited no significant difference in photostability compared with DP. On the basis of the stability data, some DP salts were a little less stable under accelerated conditions and light exposure; however, most DP salts were almost as stable as DP.

Dissolution behavior of dipyridamole salts: The preliminary dissolution testing of DP and six DP salts was carried out in 20 mL of 0.05 M phosphate buffer (pH 6.8) at 37°C using a small volume dissolution tester to confirm possible improvement of drug dissolution. The drug dissolution level of DP reached 10% of the total drug amount at 60 min because of poor DP solubility at pH 6.8 (*ca.* 6 μg/mL at 37°C) (**Table 2**).[29] In contrast, the six DP salts exhibited a higher level of dissolved drug at pH 6.8 by at least 2-fold compared with DP. In particular, the DP dissolved levels of DP/SA, DP/TS and DP/BS improved by 4.6-, 4.3- and 3.3-fold compared with DP, respectively. After dissolving DP salts, pH values of the test medium did not change from 6.8 (**Table 2**). As a result, counterions containing DP salts could modulate pH values in the diffusion area during the dissolution of DP salts, resulting in improved solubility of DP in medium at neutral pH. According to the results from stability testing, preliminary dissolution testing, DP/SA and DP/TS might be promising salt forms, although DP/SA was found to be hygroscopic, possibly leading to high variability in its potency. In this context, DP/TS was selected as a promising

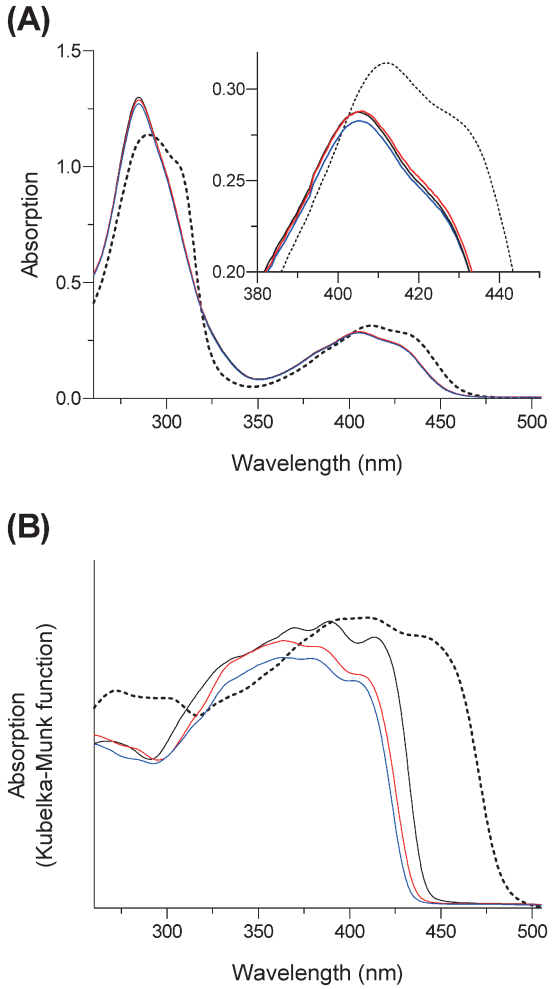

Fig. 3. UV spectral patterns of dipyridamole and dipyridamole salts
(A) UV absorption spectra and (B) UV-visible diffuse reflectance spectra. Black dotted line, DP; black solid line, DP/SA; red solid line, DP/TS; and blue solid line, DP/BS.

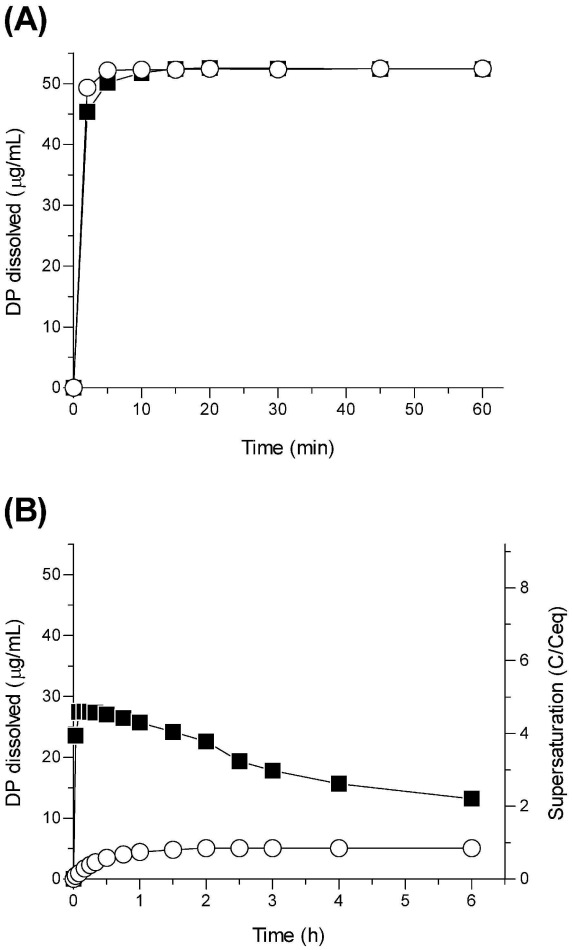

Fig. 4. Dissolution profiles of dipyridamole and dipyridamole salt at (A) pH 1.0 and (B) pH 6.8
○, DP; ■, DP/TS. Each bar represents mean ± SD of 3 independent experiments.

salt for further study. Dissolution tests of DP and DP/TS were performed in 0.1 M HCl solution (pH 1.0) and 0.05 M phosphate buffer solution (pH 6.8) using a regular pharmacopeial dissolution tester to simulate normal and hypochlorhydric conditions (**Fig. 4**). The dissolution profiles at pH 1.0 of DP and DP/TS were almost identical and reached 50 μg/mL DP concentration as almost 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).[29] At pH 6.8, DP exhibited poor dissolution behavior and a maximum dissolution level of *ca.* 5 μg/mL. In contrast, DP/TS showed rapid dissolution, and the maximum dissolved level of DP/TS was much higher than that of DP, by *ca.* 5-fold (**Fig. 4B**). The supersaturation level (C/C_{eq}) of DP/TS was found to be *ca.* 4 at 60 min, and the level seemed to gradually decrease to *ca.* 2.2 at 6 h. Thus, the supersaturation state was still maintained for at least 6 h, and no significant increase of turbidity was observed nor any precipitation. Considering these results, the newly developed DP/TS could be a promising salt and have the potential to improve oral absorption under hypochlorhydric conditions.

Pharmacokinetic profiling of dipyridamole salt: In previous clinical studies, DP showed low oral bioavailability under hypochlorhydria with increased gastric pH because DP had pH-dependent solubility.[9] In our previous studies, DP formulations with enhanced dissolution behavior at neutral pH were developed using a microenvironmental pH-modifier approach, and this formulation exhibited improved oral bioavailability in hypochlorhydric model rats.[18] In this study, some DP salts were developed, and these salts showed pH-independent dissolution behavior. This prompted us to clarify the potential improvement of oral absorption; therefore, the pharmacokinetic behaviors of DP and DP/TS were evaluated in normal rats and omeprazole-treated rats. The pharmacokinetic behavior of DP in normal rats was assessed after oral administration of DP and DP/TS (10 mg DP/kg) in order to compare it with that in hypochlorhydric model rats. The plasma concentration-time profiles of DP in rats are shown in **Figure 5**, and the related pharmacokinetic parameters are described in **Table 3**. In normal rats, the maximum concentration (C_{max}) and area under the curve of plasma concentration versus time from 0 h to 3 h (AUC_{0-3}) values of DP/TS were higher than those of DP by 2.8- and 1.7-fold, respectively; however, the AUC_{0-3} values for DP and DP/TS were not statistically different, possibly due to high variability. On the basis of the AUC value of intravenously admin-

istered DP (3.0 mg/kg),[19] absolute bioavailabilities of DP and DP/TS were calculated to be *ca.* 19% and 24%, respectively. Thus, oral bioavailabilities of DP and DP/TS were statistically identical, whereas there was significant difference in their C_{max} values. In addition to the normal rats, pharmacokinetic behaviors of DP and DP/TS were also characterized in hypochlorhydric model rats. The rats were treated with 30 mg/kg omeprazole by oral administration to prepare them as hypochlorhydric model rats. After treatment with omeprazole, the gastric pH of rats rose to about 6 within 1 h, and high gastric pH was maintained for about 5 h.[24] The pharmacokinetic behavior after oral administration of DP in omeprazole-treated rats was found to be lower than that in normal rats, and the AUC_{0-3} values in omeprazole-treated rats and normal rats were 48.4 ± 10.3 ng·h/mL and 127.7 ± 32.9 ng·h/mL, respectively. In addition, delayed oral absorption of DP was observed in omeprazole-treated rats compared with normal rats, and T_{max} values of DP in omeprazole-treated rats and normal rats were *ca.* 1.3 h and *ca.* 0.4 h, respectively. On the other hand, the pharmacokinetic behavior after oral administration of DP/TS in omeprazole-treated rats was almost identical to that in normal rats. The values of AUC_{0-3} and T_{max} in omeprazole-treated rats were 198.5 ± 21.8 ng h/mL and *ca.* 0.3 h, respectively, and did not differ significantly from those in normal rats. From the pharmacokinetic behavior of DP/TS taken together with the pH-independent dissolution behavior, salt formation could be a promising approach for improving the oral bioavailability of DP.

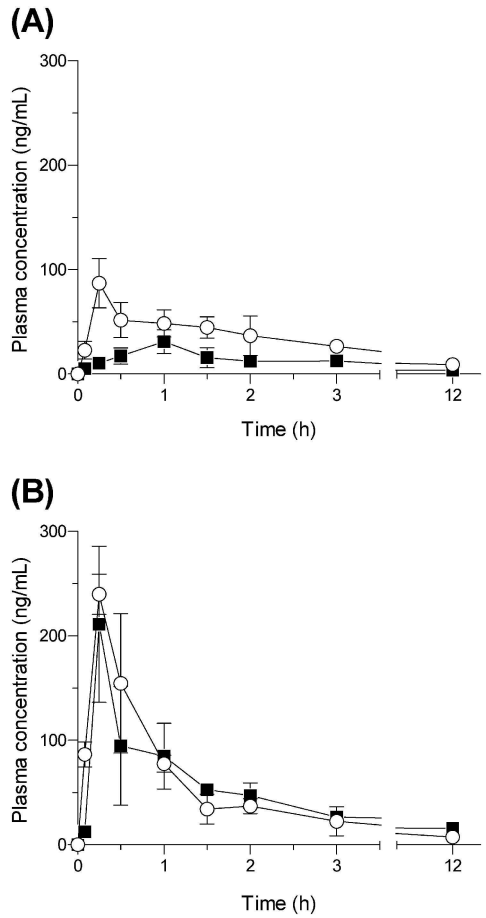

Fig. 5. Plasma dipyridamole concentrations in normal and hypochlorhydric rats after oral administration (10 mg DP/kg) of (A) DP and (B) DP/TS
○, normal rats; ■, hypochlorhydric rats. Data represent mean ± SE of 4 experiments.

In our previous studies, DP granules with 30% loading of TS (DPG/TS30) were developed using the microenvironmental pH-modifier approach, and the DPG/TS30 showed significant improvement in dissolution behavior at pH 6.8 and oral absorption in hypochlorhydric rats.[18] In both salt formation and microenvironmental pH-modifier approaches, modulation of pH value in the diffusion area led to pH-independent dissolution behavior, and these approaches could be effective to improve oral absorption for drug substances with pH-dependent solubility. In particular, salt formation could be a promising and simplified approach in pharmaceutical pre-formulation; however, sometimes it is challenging to obtain appropriate salts with expected molecular properties. For some drugs, preparation of stable salts may not be feasible, and free form may be preferred.[30] In such a case, the microenvironmental pH-modifier approach might be alternatively used in the formulation development. In addition to these solubilization strategies, a number of efficacious approaches have been developed, and, to enhance the therapeutic potential of drugs with pH-dependent solubility, careful selection of a strategic approach would be necessary on the basis of cost of goods, molecular properties and clinical needs.

In conclusion, salt screening of DP was conducted using nine counterions by the temperature gradient method, and six DP salts were obtained in the present study. All six DP salts showed improved dissolution behavior at neutral pH compared with DP. On the basis of the physicochemical properties, including dissolution behavior and stability, DP/TS was selected as a promising salt to achieve pH-independent dissolution behavior. In the pharmacokinetic study using hypochlorhydric model rats, DP/TS showed *ca.* 4-fold higher oral absorption in comparison with DP, and the pH-independent dissolution behavior of DP might explain the

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

	DP (10 mg DP/kg; *p.o.*)		DP/TS (10 mg DP/kg; *p.o.*)	
	Normal rats	Omeprazole-treated rats	Normal rats	Omeprazole-treated rats
C_{max} (ng/mL)	87.1 ± 23.6	31.1 ± 11.3	239.8 ± 19.3#	211.1 ± 74.7*
T_{max} (h)	0.38 ± 0.07	1.33 ± 0.57	0.31 ± 0.06	0.31 ± 0.06*
$T_{1/2}$ (h)	2.23 ± 0.23	2.42 ± 0.67	1.55 ± 0.64	1.22 ± 0.25
AUC (ng·h/mL)	269.2 ± 43.1	96.8 ± 25.8	341.2 ± 75.2	390.9 ± 20.9*
AUC_{0-3} (ng·h/mL)	127.7 ± 32.9	48.4 ± 10.3	213.6 ± 26.7	198.5 ± 21.8*
Oral BA (%)	18.6 ± 3.0	6.7 ± 1.8	23.5 ± 5.2	27.0 ± 1.4*
Oral BA_{0-3} (%)	10.0 ± 2.6	3.8 ± 0.8	16.7 ± 2.1	15.5 ± 1.7*

C_{max}, maximum concentration; $T_{1/2}$, half-life; AUC, area under the curve of plasma concentration; BA, bioavailability. Values are expressed as mean ± SE from 4 experiments. # $p < 0.05$ between DP and DP/TS in normal rats. * $p < 0.05$ between DP and DP/TS in omeprazole-treated rats.

enhanced oral absorption under hypochlorhydria. Salt formation is a valuable method for enhancement of dissolution behavior and oral absorption, and the newly developed DP salt might provide better therapeutic efficacy in clinical use for hypochlorhydric patients.

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