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RESEARCH PAPER

Development of Meloxicam Salts with Improved Dissolution and Pharmacokinetic Behaviors in Rats with Impaired Gastric Motility

Masanori Ochi • Ryo Inoue • Yukinori Yamauchi • Shizuo Yamada • Satomi Onoue

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ABSTRACT

Purpose Because of its poor solubility in acidic solution, oral absorption and efficacy of meloxicam (MEL) may be reduced in severe pain patients with impaired gastric motility. The present study aimed to develop salt forms to overcome these drawbacks.

Method Upon MEL salt screening with eight counterions, five MEL salts were obtained. The physicochemical properties of these MEL salts were characterized with a focus on morphology, crystallinity, thermal behavior, dissolution, and chemical/photostability. Pharmacokinetic profiling of an orally administered MEL salt was also carried out in both normal rats and rats treated with propantheline for the suppression of gastric motility.

Results Dissolution behaviors for all obtained MEL salts were markedly better than that of crystalline MEL; in particular, the initial dissolution rate of arginine MEL dihydrate (MEL/Arg) was ca. 14-fold higher than that of crystalline MEL. MEL/Arg was found to be chemically and physically stable. There was ca. 18-fold reduction of AUC_{0-4} for orally dosed crystalline MEL (1.0 mg-MEL/kg) in propantheline-treated rats compared with that in normal rats. In contrast, there was only a ca. 3-fold difference in AUC_{0-4} between normal and propantheline-treated rats after oral administration of MEL/Arg (1.0 mg-MEL/kg).

Conclusion From these findings, MEL/Arg may provide improved oral absorption in severe pain patients.

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KEYWORDS Absorption · dissolution · meloxicam · salt screening · stability

ABBREVIATIONS

ANOVA	analysis of variance
Arg	L-arginine
AUC	area under the curve of plasma MEL concentration *versus* time
C_{max}	maximum concentration
DEA	diethanolamine
DTA	differential thermal analyses
Gly	glycine
Lys	lysine
MEL	meloxicam
NMR	nuclear magnetic resonance
NSAID	non-steroidal anti-inflammatory drug
PLM	polarized light microscopy
RH	relative humidity
SD rats	Sprague-Dawley rats
SEM	scanning electron microscopy
TEA	triethanolamine
TGA	thermogravimetric analysis
T_{max}	time to maximum concentration
Tris	tris(hydroxymethyl)aminomethane
UPLC/ESI-MS	ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry
UV	ultraviolet
XRPD	X-ray powder diffraction

INTRODUCTION

Meloxicam (MEL), 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2- benzothiazine-3-carboxamide-1,1-dioxide, is a non-steroidal anti-inflammatory drug (NSAID) used for

the clinical treatment of rheumatoid arthritis (1), osteoarthritis (2), and postoperative pain (3). MEL exhibits higher analgesic and anti-inflammatory activities with less ulcerogenic potency than other NSAIDs (1). Despite this attractive pharmacological profile, crystalline MEL has low solubility in acidic solution (ca. 0.6 μg/mL at both pH 1.2 and 4.0) (4); therefore, the oral absorption behavior of crystalline MEL may be influenced by gastric motility. It is well established that gastric motility tends to be impaired in severe pain patients (5,6). Thus, the drug efficacy of crystalline MEL early after oral administration may be limited in severe pain patients and result in subsequent limited systemic exposure of MEL early after oral administration. To overcome these drawbacks, improvements in the dissolution behavior of MEL in gastric fluid are essential.

There are many efficacious techniques to increase the aqueous solubility of poorly soluble drugs, such as salt formation (7), micronization (8), the solid dispersion technique (9), and emulsification (10). In particular, salt formation has been widely used to improve aqueous solubility because of its high pharmaceutical developability, such as low toxic potential and ease of manufacturing (7). Approximately 40% of orally administered drugs were developed as drug salts for improving physicochemical and pharmacokinetic properties (11). In a previous study, dissolution in acidic solution and *in vivo* oral absorption rates of crystalline MEL in normal rats were improved via salt formation with ethanolamines (12). There were marked improvements in both dissolution and pharmacokinetic behaviors of MEL via salt formation with ethanolamines, although chronic use of ethanolamines was not preferable because of their hepatic accumulation and hepatocarcinogenic potential (13,14). In this context, further development of MEL salts with high safety has been required; however, salt screening for MEL salts has not been fully demonstrated with a focus on physicochemical stability and improved pharmacokinetic behavior of MEL salts in severe pain patients with impaired gastric motility.

The present study aimed to develop a novel MEL salt with improved dissolution in acidic solution, and to increase the pharmacological effects of MEL in severe pain patients using the MEL salt. In this study, the physicochemical properties of five MEL salts, including two previously reported MEL salts, were characterized by polarized light microscopy (PLM) and scanning electron microscopy (SEM) observations for morphology, X-ray powder diffraction (XRPD) analysis for crystallinity, thermogravimetric and differential thermal analyses (TG/DTA), dissolution testing, and stability testing. From these findings, the most suitable MEL salt for *in vivo* testing was selected in view of its dissolution profile and physicochemical stability. Pharmacokinetic profiling after oral administration of the MEL salt was carried out in both normal rats and rats treated with propantheline for the suppression of gastric motility.

MATERIALS AND METHODS

Materials

Sodium hydroxide (NaOH), potassium hydroxide (KOH), tris(hydroxymethyl)aminomethane (Tris), L-arginine (Arg), glycine (Gly), lysine (Lys), diethanolamine (DEA), and triethanolamine (TEA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Crystalline MEL (Fig. 1) was supplied from Boehringer Ingelheim Japan Co., Ltd. (Kobe, Japan). All other chemicals were purchased from commercial sources.

Preparation of MEL Salts

MEL salts were prepared by a temperature gradient technique. Briefly, 175.7 mg of MEL and an equimolar amount of NaOH, KOH, Arg, Tris, Gly, Lys, DEA, or TEA solution (400 mM) were dissolved in a minimal volume of crystallization solvent (ethanol, acetone, 1-propanol, and 2-propanol) at 60°C. Then, each MEL-counterion solution was cooled at 4°C overnight. Precipitates were collected by suction filtration, and each filtrate was washed with crystallization solvent several times. Solid filtrates were dried in a vacuum overnight.

PLM Observation

Representative PLM images of MEL samples were taken using a CX41 microscope (Olympus Co. Ltd., Tokyo, Japan). MEL samples were examined under various conditions including differential interference contrast, slightly uncrossed polars, and using a red wave compensator.

SEM Observation

Each sample was coated with platinum on a HITACHI Ion Sputter E-1010 (Hitachi, Tokyo, Japan). Representative SEM images of MEL samples were taken using VE-7800 (Keyence Co., Ltd., Osaka, Japan). For SEM observations, each sample was fixed on an aluminum sample holder using double-sided carbon tape.

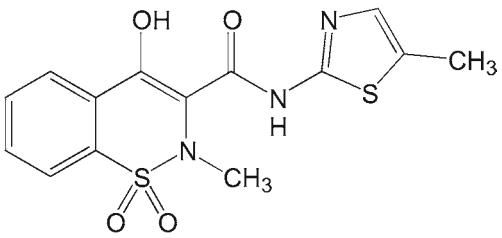

Fig. 1 Chemical structure of meloxicam (MEL).

XRPD Analysis

The XRPD 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 a scanning speed of 6°/min.

TG/DTA

TG/DTA was performed using a Rigaku Thermo plus TG8120 (Rigaku Co., Ltd., Tokyo, 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 5°C/min with nitrogen purge at 40 mL/min. The temperature axis was calibrated with indium (8–10 mg, 99.999% pure, onset at 156.6°C).

Nuclear Magnetic Resonance (NMR) Analysis

^{1}H-NMR spectra of MEL samples were recorded on a JEOL ECA 500 spectrometer (JEOL Ltd., Tokyo, Japan) using dimethylsulfoxide-d_6 (99.9% D, Wako Pure Chemical Industries, Ltd.) as a solvent.

Determination of Water Content in MEL Salts

The water content amount in MEL salts was determined using an MKA-520 (Karl Fischer Moisture Titrator, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan).

UPLC Analysis

The concentration of MEL was determined by ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry (UPLC/ESI-MS) analysis. The UPLC/ESI-MS system consisted of Waters Acquity UPLC system (Waters, Milford, MA, USA), which included a binary solvent manager, sample manager, column compartment, and SQ detector connected with Waters Masslynx version 4.1. Waters Acquity UPLC BEH C18 (particle size: 1.7 μm, column size: 2.1 mm×50 mm) was used. Column temperature was maintained at 40°C, and samples were separated using a gradient mobile phase consisting of Milli-Q containing 0.1% formic acid (A) and methanol (B). The gradient condition of the mobile phase was 0–0.5 min, 70% A; 0.5–3.0 min, 70–20% A; 3.0–4.0 min, 5% A, and the flow rate was set at 0.25 mL/min. Analysis was carried out using selected ion recording (SIR) for specific m/z 350 for MEL $[M\text{-}H]^-$. The capillary voltage was set at 3.1 kV with a cone voltage of 30 V.

Solid State Stability Study

MEL samples were stored at 40±2°C/75±5% relative humidity (RH) for 4 weeks in a stability chamber (Labcare Pvt. Ltd., Mumbai, India). Samples after storage were subjected to XRPD analysis, TG/DTA, and purity analysis by UPLC/ESI-MS.

Photostability Study

For solid-state photostability testing, each MEL sample (2 mg) was weighed exactly and spread in a 1.5 mL clear glass vial (12 mm×32 mm, Shimadzu Co., Ltd., Kyoto, Japan) over the entire bottom surface. MEL salts were set in the Suntest CPS+ solar simulator (Atlas Material Technology LLC, Chicago, IL, USA) and irradiated with ultraviolet (UV) and visible light (900 kJ/m^2), and the remaining amount of MEL in samples was determined by UPLC/ESI-MS as described in the "UPLC Analysis" section. UV and visible light irradiation was carried out at 25°C with an irradiance of 250 W/m^2. Reference samples under the protection of aluminum foil were examined under the same conditions.

Dissolution Test

Dissolution tests were carried out for 120 min in 900 mL of HCl solution (pH 1.2) using the rotating basket method with constant stirring at 50 rpm in a dissolution test apparatus NTR 6100A (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 37°C. Each MEL sample was weighed to keep the total amount of MEL in the dissolution vessel constant at 10 mg. Analyzed samples were collected at the indicated times (10, 20, 30, 60, 90, and 120 min) with a Toyama W-PAS-615 auto sampler (Toyama Sangyo Co., Ltd., Osaka, Japan), and were centrifuged at 10,000 g for 5 min. Supernatants were diluted with an equal volume of methanol, and the concentrations of MEL were determined by Waters UPLC/ESI-MS as described in the "UPLC Analysis" section.

Animal Studies

Male Sprague-Dawley (SD) rats (8–9 weeks of age; 239.6–278.4 g in weight; Japan SLC, Shizuoka, Japan) were housed two per cage in the laboratory with free access to food and water, and were 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.

Male SD rats were fasted for 18 h prior to the experiment but were given free access to water. A dose equivalent to

1.0 mg-MEL/kg body weight was administered orally to both normal rats and propantheline-treated rats with suppressed gastric motility. Orally administered suspensions were prepared with distilled water and the dosing concentration was 0.5 mg-MEL/mL. Intraperitoneal administrations of 20 mg/kg propantheline suspension (20 mg/kg body weight) were conducted 2 and 1 h prior to MEL administration to achieve vagal suppression in accordance with a previous report (15). Blood samples were collected from the femoral artery at 0, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, and 24 h after oral administration. Each blood sample was centrifuged at 10,000×*g* for 5 min to prepare plasma samples. Plasma MEL concentrations were determined by UPLC/ESI-MS. Briefly, 150 μL of methanol was added to 50 μL of plasma sample, and this was then centrifuged at 3,000 rpm for 10 min. All samples were stored at −20°C without light exposure until they were analyzed.

RESULTS AND DISCUSSION

Preparation of MEL Salts and Their Characterization

In this study, counterions such as Na, K, Tris, Arg, Gly, and Lys were selected for salt screening because of their low toxic potential and clinical usage as orally administered ingredients (11,16,17). Ethanol, 1-propanol, and acetone were used as crystallization solvents given their less toxic potential according to the ICH guidelines for residual solvents (18). For salt screening, PLM experiments were carried out to evaluate the crystallinity of precipitates from MEL-counterion solutions. Of all tested samples, birefringence was observed in precipitates from MEL-K ethanol solution (MEL/K), MEL-Arg 1-propanol solution (MEL/Arg), MEL-Tris 1-propanol solution (MEL/Tris), MEL-DEA ethanol solution (MEL/DEA), and MEL-TEA acetone solution (MEL/TEA), indicating crystal formation in these precipitates. Yields of these MEL samples were over 70% using a temperature gradient method. In contrast, crystalline precipitates were not obtained from MEL-Na, MEL-Gly, and MEL-Lys solutions. Morphological differences among crystalline precipitates were observed by SEM, which revealed a plate-like morphology of MEL/TEA, needle-like morphology of MEL/K, MEL/Arg, MEL/Tris, and MEL/DEA, and rock-like morphology of crystalline MEL (Fig. 2b, Table I). The appearances of MEL/K and MEL/DEA were similar and were far smaller than those of MEL/TEA and MEL/Arg. To evaluate crystal forms of MEL samples, XRPD analysis and TG/DTA were conducted (Fig. 3). Distinguishing XRPD patterns of MEL samples were observed, and there were significant differences in XRPD patterns among MEL salts, suggesting different crystal forms. TG/DTA indicated that endothermic peak onset temperatures (T_{on}) of prepared MEL/DEA and MEL/TEA were 178 and 212°C, respectively (Fig. 4a), which corresponded well to a previous observation (19). TG/DTA showed endothermic weight loss in MEL/K (T_{on}: 145°C), MEL/Tris (T_{on}: 119°C), and MEL/Arg (T_{on}: 83°C), suggesting the possible formation of hydrate or solvate. To characterize MEL/K, MEL/Arg, and MEL/Tris in more detail, ^{1}H-NMR and/or Karl Fischer titration analyses were conducted (data not shown). According to ^{1}H-NMR analysis, 1-propanol was detected in MEL/Tris, and the molar ratio of MEL, Tris, and 1-propanol was approximately 2:2:1. The endothermic weight loss of MEL/Tris of 6.4% from 25 to 150°C (Fig. 4a and b) also almost corresponded to the theoretical content of 1-propanol (6.0%) in 1-propanol hemisolvate of Tris MEL. In contrast, crystallization solvents such as ethanol and 1-propanol were negligible in MEL/K and MEL/Arg as determined by ^{1}H-NMR analysis. The molar ratio of Arg to MEL in MEL/Arg was approximately 1:1 according to the results from ^{1}H-NMR (1.00:0.97) and UPLC/ESI-MS analyses (1.00:0.98). Karl Fischer titration analysis indicated that the water content of MEL/Arg was 6.9%, which corresponded to an endothermic weight loss of MEL/Arg of 6.9% from 25 to 125°C (Fig. 4a and b), suggesting hydrate formation in MEL/Arg, and the molar ratio of MEL, Arg, and H_2O was deduced to be 1:1:2. Karl Fischer titration analysis demonstrated 4.5% water content in MEL/K, which was consistent with the endothermic weight loss of MEL/K by 4.4% from 25 to 175°C (Fig. 4a and b). From these results, the molar ratio of MEL, K, and H_2O was deduced to be 1:1:1. Thus, 3 new (MEL/K, MEL/Tris, and MEL/Arg) and 2 previously reported MEL salts (MEL/DEA and MEL/TEA) were successfully obtained. According to the results from TG/DTA, ^{1}H-NMR, Karl Fischer titration, and UPLC/ESI-MS analyses, MEL/K, MEL/Tris, and MEL/Arg were deduced to be monohydrate, 1-propanol hemisolvate, and dehydrate, respectively (Table I).

Physicochemical Properties of MEL Salts

Dissolution property in acidic solution is important for improving reduced pharmacological effects in severe pain patients, since gastric motility could be impaired in patients with severe pain. However, crystalline MEL exhibited a poor dissolution rate in acidic solution, and the amount of dissolved MEL from crystalline MEL was 0.09 μg/mL at 120 min with an initial dissolution rate constant of 5.3×10^{-3} h^{-1} (Fig. 5, Table I). In contrast, all tested MEL salts showed improved dissolution behaviors, dissolved MEL from MEL/K, MEL/Tris, MEL/TEA, MEL/DEA, and MEL/Arg at 120 min was found to be ca. 0.33, 0.49, 0.51, 0.65, and 0.85 μg/mL, respectively. On the basis of the reported solubility of MEL at pH 1.2 (ca. 0.6 μg/mL)

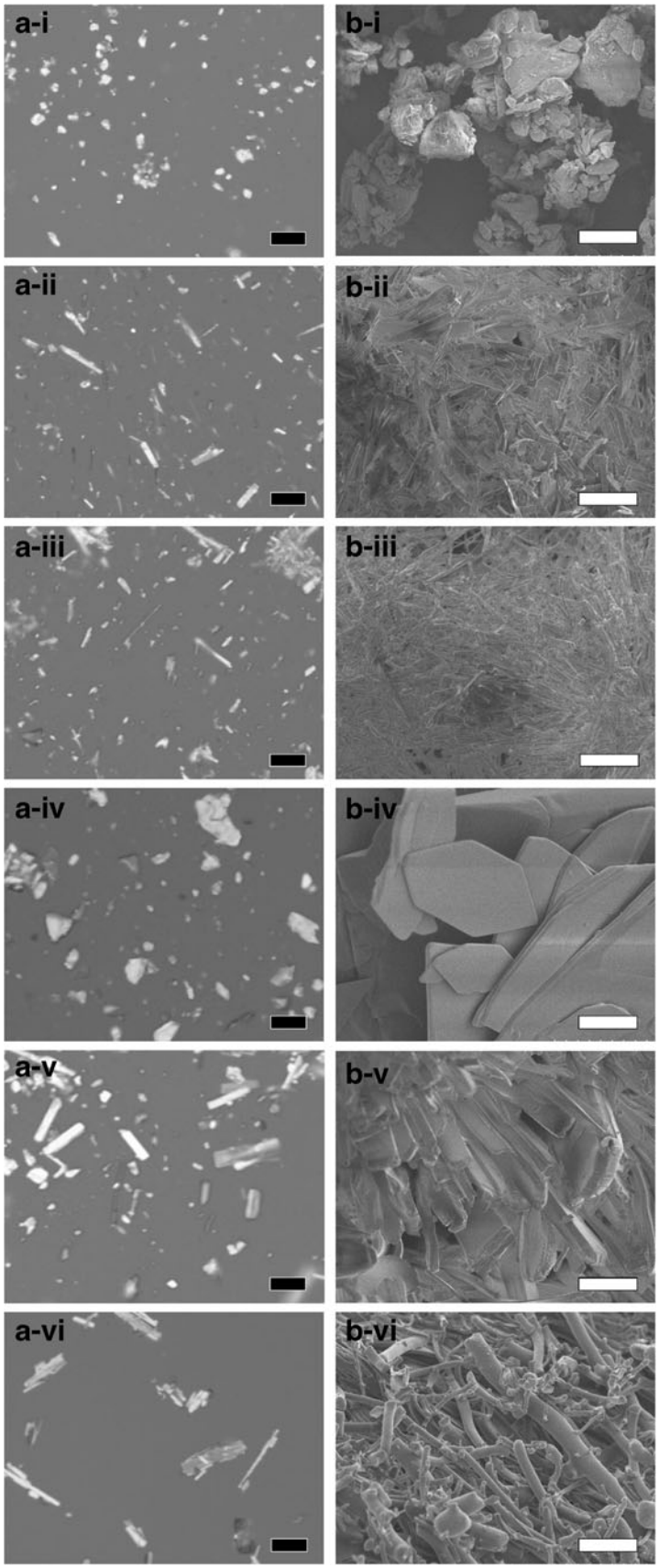

Fig. 2 Micrographic images of MEL samples. (**a**) Polarized light microscopy and (**b**) scanning electron microscopy observations. (i) MEL, (ii) MEL/K, (iii) MEL/DEA, (iv) MEL/TEA, (v) MEL/Tris, and (vi) MEL/Arg. Black and white bars represent 20 and 10 μm, respectively.

Table I Physicochemical Properties of MEL Salts

	MEL	MEL salts				
		K	Tris	Arg	DEA	TEA
Morphology	Rock-like	Needle-like	Needle-like	Needle-like	Needle-like	Plate-like
Crystallization solvent	–	Ethanol	1-Propanol	1-Propanol	Ethanol	Acetone
Stoichiometry	–	1: 1	1: 1	1: 1	1: 1	1: 1
Solvate formation	Anhydrous	Monohydrate	Hemisolvate (1-Propanol)	Dihydrate	Anhydrous	Anhydrous
Initial dissolution rate constant at pH 1.2 (h^{-1})	0.5×10^{-2}	2.8×10^{-2}	3.7×10^{-2}	7.6×10^{-2}	6.4×10^{-2}	3.7×10^{-2}
Stability (40°C/75% RH for 4 weeks) Physical stability [a]	Stable	Stable	Unstable (Polymorph)	Stable	Stable	Unstable (Polymorph)
Chemical stability	Stable [c]	Stable [c]	Stable [c]	Stable[c]	Stable [c]	Stable [c]
Photostability[b]	Stable [c]	Stable [c]	Stable [c]	Stable [c]	Stable [c]	Stable [c]

[a] Stability of crystal form before and after storage. [b] Changes in MEL purity before and after UV and visible light irradiation (250 W/m^2 for 60 min). [c] Less than 3% reduction in purity.

(4), dissolved amounts of MEL from MEL/Arg and MEL/DEA were higher than the solubility of MEL. These observations were consistent with previous reports (20), showing that the Arg salt of a poorly soluble drug led to a marked improvement in the dissolution behavior. Initial dissolution rate constants of MEL from MEL/K, MEL/Tris, MEL/TEA, MEL/DEA, and MEL/Arg were calculated to be 2.8×10^{-2}, 3.7×10^{-2}, 3.7×10^{-2}, 6.4×10^{-2}, and 7.6×10^{-2} $h,^{-1}$ respectively (Table I). Thus, the initial dissolution rates of all tested MEL salts were found to be over 5-fold higher than that of crystalline MEL. In particular, the initial dissolution rate of MEL/Arg was found to be ca. 14-fold higher than that of crystalline MEL. Previously, Serajuddin suggested that a quantitative trend exists where the solubility increases with an increase in anion or cation charge and decreases with an increase in ionic radius (7), and the outcomes from present study were partly in agreement with the previous empirical data. Improved dissolution behaviors of the drug salt of a polyvalent counterion relative to that of the drug salt of a monovalent counterion were also observed previously (21). Mechanisms for improved dissolution rates

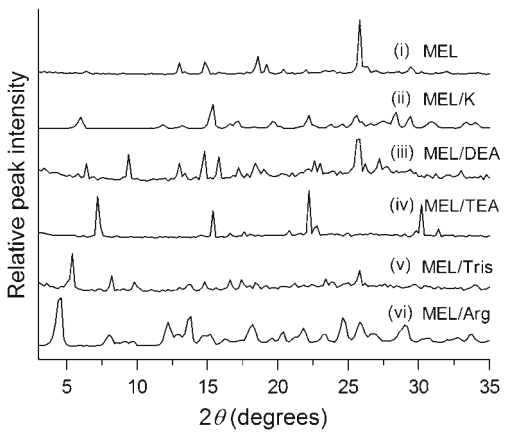

Fig. 3 X-ray powder diffraction patterns of MEL samples. (i) MEL, (ii) MEL/K, (iii) MEL/DEA, (iv) MEL/TEA, (v) MEL/Tris, and (vi) MEL/Arg.

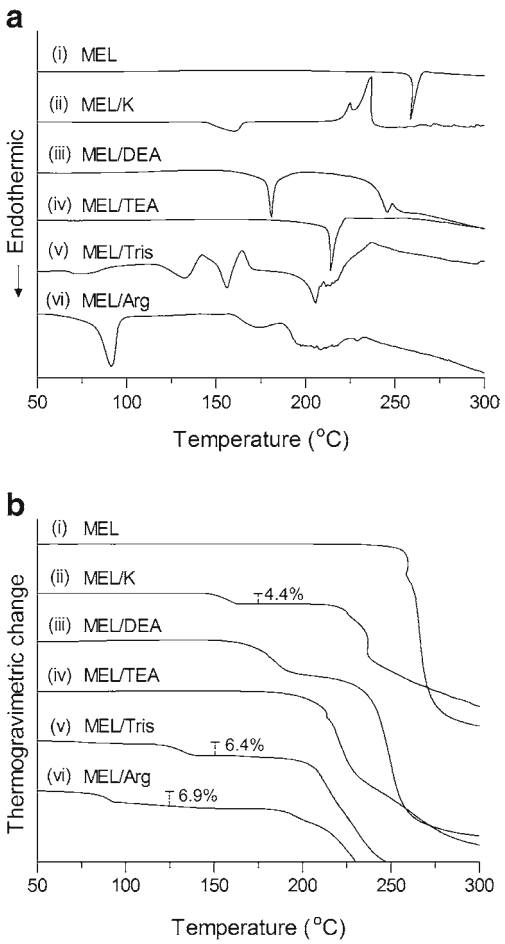

Fig. 4 Thermograms of MEL samples by (**a**) differential thermal analysis and (**b**) thermogravimetric analysis. (i) MEL, (ii) MEL/K, (iii) MEL/DEA, (iv) MEL/TEA, (v) MEL/Tris, and (vi) MEL/Arg.

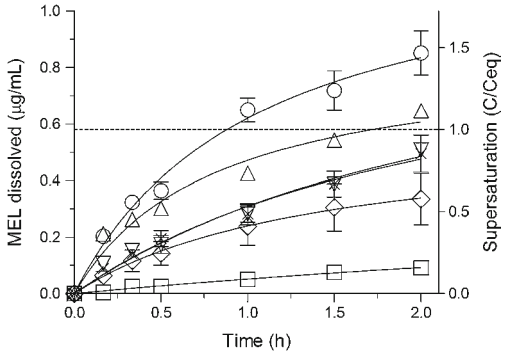

Fig. 5 Dissolution profiles of MEL samples in acidic solution (pH 1.2). □, MEL; ◇, MEL/K; ×, MEL/Tris; ▽, MEL/TEA; △, MEL/DEA; and ○, MEL/Arg. Degrees of supersaturation are expressed as measured concentration of dissolved MEL (C) *versus* equilibrium solubility of MEL (Ceq). Each bar represents mean±SE (n=3).

were thought to involve the buffering effect of a polyvalent ion at the diffusion layer (21). Thus, the reason for the highest improvement in the dissolution behavior of MEL/Arg may be that it had the highest buffering effect in the diffusion layer in all prepared MEL salts.

High physicochemical stabilities of drugs could be indispensable for sufficient therapeutic efficacy and safe therapy. In the present study, MEL samples were stored at 40°C/75% RH for 4 weeks and physicochemical stabilities of MEL samples before and after storage were studied with a focus on purity and crystal form. UPLC/ESI-MS analysis demonstrated no significant reductions in MEL (<3%) in all tested MEL samples after 4 weeks of storage (Table I). Although reductions in MEL purity of MEL salts were negligible, crystal forms could be changed, possibly leading to slow dissolution and delayed absorption. To clarify possible changes in the crystal form, TG/DTA and XRPD analyses were carried out on MEL samples. According to TG/DTA, the thermal behaviors of crystalline MEL, MEL/K, MEL/DEA, MEL/TEA, and MEL/Arg did not differ significantly between before and after storage. No significant XRPD pattern differences were observed by XRPD analysis in crystalline MEL, MEL/K, MEL/DEA, and MEL/Arg before and after storage at 40°C/75% RH for 4 weeks, indicating the high physicochemical stabilities of these MEL samples. In contrast, XRPD patterns of MEL/Tris and MEL/TEA were changed after storage, indicating crystal polymorphic changes. TG/DTA of MEL/Tris after storage demonstrated the possibility of hydrate formation. The present stability testing under the accelerated condition was undertaken for screening purposes; therefore, further stability testing for much longer periods would be needed to clarify long-term stabilities of MEL salts.

The photochemical properties of a drug salt could be significantly different from those of a free drug, as observed for the citrate salt of tamoxifen (22), possibly leading to photodegradation of the active ingredient and generation of toxic photodegradants; as such, the photostability of a drug salt needs to be evaluated for sufficient therapy without undesired side effects. For evaluation of the solid-state photostability of MEL salts, crystalline MEL samples were irradiated with UV and visible light with a total energy of 900 kJ/m^2, and the amounts of MEL remaining in the MEL samples were determined by UPLC/ESI-MS. No significant degradations in MEL (<3%) of all tested MEL samples after UV and visible light exposure were observed by UPLC/ESI-MS analysis (Table I), suggesting high photostabilities of all tested MEL salts under these conditions.

The physicochemical properties of tested MEL salts are summarized in Table I, and salt selection for *in vivo* testing was conducted on the basis of matrix decision-making. The order of increasing initial dissolution rates of MEL salts was MEL/K, MEL/TEA, MEL/Tris, MEL/DEA, and MEL/Arg. Dissolved amounts of MEL from MEL/Arg and MEL/DEA were higher than the intrinsic solubility of MEL at pH 1.2. Crystal forms of MEL/Tris and MEL/TEA were changed after storage at 40°C/75% RH for 4 weeks according to XRPD analysis and TG/DTA before and after storage. In contrast, purity, crystal form, and thermal behaviors of crystalline MEL, MEL/K, MEL/DEA, and MEL/Arg were not changed after storage at 40°C/75% RH for 4 weeks. Although the initial dissolution rate of MEL/DEA was the highest next to MEL/Arg, DEA has hepatocarcinogenic and high hepatic accumulation potentials, so the chronic toxicity potential of orally taken DEA may not be low enough for chronic use. Taking into account the physicochemical properties of MEL salts and toxicological properties of counterions tested, MEL/Arg would be the most suitable salt for *in vivo* testing among the MEL salts tested.

Pharmacokinetic Study

Plasma concentration-time profiles of MEL in normal rats after oral administration of crystalline MEL or MEL/Arg suspension (1 mg-MEL/kg body weight) in both normal and propantheline-treated rats are shown in Fig. 6a and b, respectively, and relevant pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-4}, and AUC_{0-24} were listed in Table II. Oral administration of crystalline MEL to normal rats resulted in gradual elevations in plasma MEL concentrations up to C_{max} of 1.1 μg/mL with T_{max} of 4.3 h. In contrast, the pharmacokinetic behavior of MEL in MEL/Arg was better than that in crystalline MEL. Oral administration of MEL/Arg resulted in rapid elevations of plasma MEL levels up to C_{max} of 2.2 μg/mL with T_{max} of 2.2 h. Thus, upon salt formation with Arg, C_{max} and AUC_{0-4} of MEL were 2.0- and 2.4-fold higher, respectively, and T_{max} was likely to be shorter than that of crystalline MEL-administered rats. Since rapid onset of action is required for an analgesic drug, MEL/Arg could contribute to more

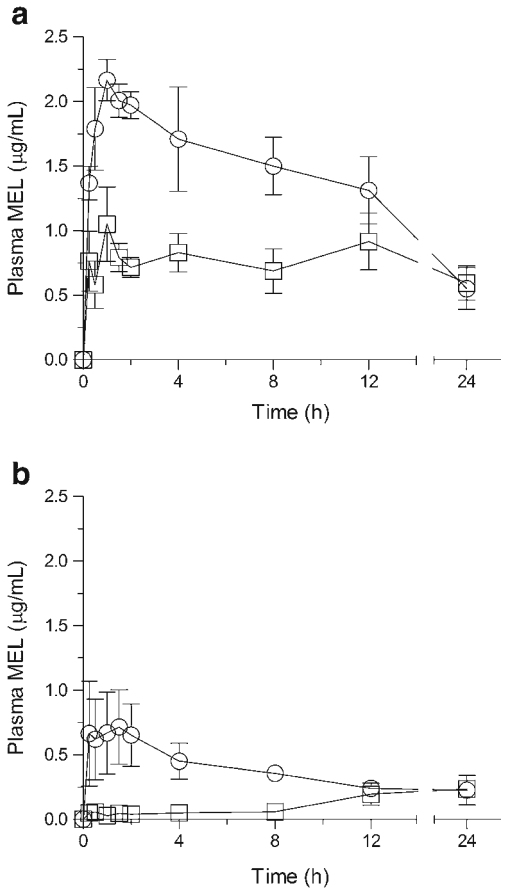

Fig. 6 Plasma MEL concentration-time profiles in (**a**) normal and (**b**) propantheline-treated rats after oral administration of MEL samples. ○, MEL/Arg; and □, MEL (1.0 mg-MEL/kg body weight). Each bar represents mean±SE (n=3–4).

efficacious treatment of acute pain. In the previous study, orally dosed MEL/DEA and MEL/TEA also exhibited a trend toward the increase in AUC_{0-4} by 1.6- and 1.5-fold, respectively, implying the faster absorption of these MEL salts (12). On the basis of these pharmacokinetic data, as well as the toxic potential of ethanolamines (13,14), the MEL/Arg might exceed MEL ethanolamine salts in the therapeutic value.

Although MEL has attractive pharmacological activities, the absorption and onset of action of MEL could be delayed in patients with severe pain, as observed for other NSAIDs with poor solubility in acidic solution (15,23). Delayed absorption could be attributed to suppression of the vagal nervous system, leading to a reduction in both gastric motility and excretion of gastric fluid (23). Jamali previously demonstrated that vagal suppression in rats was achieved by intraperitoneal injection of propantheline solution (20 mg-propantheline/kg), and vagally suppressed rats appeared to be a useful animal model for testing the absorption of drugs that were compromised by pain and/or its associated trauma (15). In the present study, MEL/Arg was orally administered to propantheline-treated rats with suppressed gastric motility for the evaluation of reduced incomplete absorption. Significant reductions in systemic exposure of MEL were observed early after oral administration of crystalline MEL in propantheline-treated rats (Fig. 6b), as evidenced by AUC_{0-4} and T_{max} of MEL being 18-fold lower and 4-fold longer, respectively, in propantheline-treated rats than those of normal rats. These observations were in agreement with the outcomes from previous pharmacokinetic studies on MEL in normal and vagally suppressed rats (24). In contrast, impaired absorption of MEL early after oral administration was better in MEL/Arg-administered rats than that in crystalline MEL-administered rats, as evidenced by an only 3-fold difference in AUC_{0-4} between normal and propantheline-treated rats after oral administration of MEL/Arg. Thus, MEL/Arg could improve systemic exposure early after oral administration in propantheline-treated rats. These findings were consistent with the results of a dissolution test in acidic solution, demonstrating that the dissolution rate of MEL/Arg was markedly higher than that of crystalline-MEL. According to the biopharmaceutics classification system (BCS) (25), MEL was categorized into BCS class II (26), the characteristics of which are identified as low solubility and high membrane permeability. Generally, the bioavailabilities of BCS class II drugs are rate-limited by their dissolution, and a small increase in dissolution profile

Table II Pharmacokinetic Parameters of MEL Salts following Oral Administration in Normal and Propantheline-Treated Rats

	MEL		MEL/Arg	
	Normal rats	Propantheline-treated rats	Normal rats	Propantheline-treated rats
C_{max} (μg/mL)	1.1±0.3	0.3±0.1	2.2±0.1	0.9±0.3
T_{max} (h)	4.3±2.7	18.0±4.0	2.2±0.9	2.5±1.9
AUC (μgh/mL)				
0–4 h	3.1±0.2	0.2±0.1 (5.6%)[a]	7.3±0.4	2.4±1.0 (32.4%)[a]
0–24 h	18.3±1.3	3.5±0.8 (19.1%)[a]	30.5±2.0	8.0±2.5 (26.2%)[a]

C_{max}: maximum concentration; T_{max}: time to maximum concentration; AUC: area under the curve of plasma MEL concentration *vs.* time from 0 to *t* h after oral administration. Data represent mean±SE (n=3–4). [a], Ratio of AUC values between normal and propantheline-treated rats.

sometimes results in a large increase in bioavailability (27). These previous findings suggested a marked increase in the solubility of MEL may be attributable to a reduction in delayed absorption and onset of action. Generally, since rapid onset of action is required for the treatment of acute exacerbations of rheumatism, salt formation of MEL with Arg could contribute to more efficacious oral dosage forms in patients with severe pain, possibly leading to improved clinical outcomes. However, the use of conventional tablets and capsules for MEL/Arg may result in delayed absorption and slow onset of action. For efficient absorption of a drug that is incorporated in a solid dosage form, rapid disintegration and dissolution are essential as both of these processes are facilitated by physical motion and the presence of fluid. Since both of these factors seem to be depressed by vagal suppression, pharmacokinetic behaviors and clinical outcomes may be variable depending on the dosage form in severe pain patients.

Previously, Jamali and co-workers attempted to improve systemic exposure of MEL in propantheline-treated rats using the fast-dissolving formulation containing oil surfactant, Gelucire 44/14 (24). Similar to our outcomes, the dissolution behavior of MEL in acidic solution was improved. C_{max} and AUC_{0-24} of MEL were slightly lower in propantheline-treated rats than those in normal rats, although the toxic potential of Gelucire 44/14 was not acceptable for chronic use because treatment of Caco-2 cells with Gelucire 44/14 resulted in decreased cell viability and reduced expression and activity of P-glycoprotein (28). In addition to MEL salts, ibuprofen salts such as sodium dehydrate and arginate also have a faster onset of pain relief in patients with postoperative dental pain, than that of a conventional ibuprofen formulation (29,30). In particular, ibuprofen sodium dehydrate could reduce the intensity of pain by 50% 30 min earlier and provide substantial pain relief 14 min earlier, and these findings would provide further support for the effectiveness of the salt formation in improving the therapeutic potential of NSAIDs in pain patients. Since pain treatment often requires long-term therapy and causal treatment, the toxic potential of inactive ingredients could be a serious drawback. In contrast, consumption of MEL/Arg equivalent to the maximum recommended daily dose of MEL in humans would result in the consumption of 7.4 mg of Arg. This dose of Arg would be safe for human use, considering that the dietary allowance of Arg is 4,700–5,400 mg and Arg has been administered intravenously at doses of 400 mg/day (31,32). Safety information, taken together with dissolution and pharmacokinetic behaviors, suggested that newly developed MEL/Arg may provide safe medication with rapid onset of analgesic action.

CONCLUSION

In the present study, five MEL salts were prepared using a temperature gradient method and their physicochemical and pharmacokinetic properties were characterized. There were significant improvements in dissolution behaviors of all tested MEL salts in acidic solution; in particular, MEL/Arg exhibited ca. 14-fold higher initial dissolution rate at pH 1.2 than crystalline MEL. TG/DTA, NMR, and UPLC/ESI-MS analyses indicated that MEL/Arg was the dihydrate of arginine MEL. There was no significant reduction or crystalline polymorph in MEL/Arg after storage at 40° C/75% RH for 4 weeks and after UV and visible light irradiation (250 W/m^2 for 60 min). Systemic exposure of MEL early after oral administration with MEL/Arg in propantheline-treated rats was better than that with crystalline MEL. From these findings, MEL/Arg may be a simple and safe method of achieving the pharmacological action of MEL in severe pain patients.

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