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Research Paper

Formulation of solid dispersion of rebamipide evaluated in a rat model for improved bioavailability and efficacy

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Abstract

Objectives Rebamipide, a novel anti-ulcer agent, is listed in biopharmaceutics classification class IV because of its low aqueous solubility and permeability. Consequently, the bioavailability of rebamipide is under 10% in humans. The aim of this study was to increase the solubility and determine the effect of solubility enhancement on the bioavailability and efficacy of rebamipide (RBM).

Methods After taking into account the physiochemical properties of RBM (solubility, melting point, dosage etc.), solid dispersion was chosen as the solubility enhancement method. A rebamipide solid dispersion system containing the drug, L-lysine, PVP-VA 64 and poloxamer 407 was obtained from a spray-drying method. Solubility enhancement of RBM from the solid dispersion was determined by a dissolution test in 900 ml at pH 1.2. The bioavailability and efficacy of RBM solid dispersion were evaluated in a rat model.

Key findings The aqueous solubility of RBM was improved 62.17 times by solid dispersion. The oral bioavailability of the drug was also increased 1.74-fold from solid dispersion compared with the reference product in a rat model. With regard to the anti-ulcer effect, the percentage inhibition of the solid dispersion was 2.71 times higher than that of the reference product in the ulcer-induced rat model.

Conclusions A solid dispersion of rebamipide was successfully formulated using the spray-drying method. Bioavailability and efficacy of rebamipide were increased significantly by solubility enhancement of the drug.

Keywords pharmacodynamic; pharmacokinetic; rebamipide; solid dispersion

Introduction

Rebamipide [2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl] proponoinc acid] (RBM), a novel quinolinon derivative, is a potent antiulcer agent with its main pharmacological actions being mediated by increasing endogenous prostaglandin synthesis, scavenging oxygen-derived free radicals, and decreasing neutrophil activity and inflammation processes.[1] The main indication of RBM is the treatment of gastric ulcers, acute gastritis and exacerbated chronic gastritis.

RBM is known to possess low aqueous solubility and poor oral absorption, thus it is listed in biopharmaceutics classification system IV. The oral bioavailability of this drug is very low at around 10%, therefore RBM is used as a model drug to plan different strategies for improving bioavailability. Most research activity focused on bioavailability involves the use of certain enhancers. Sodium laurate and taurine have been used to improve rectal absorption of rebamipide.[2] The combination of spermine and sodium taurocholate increases bioavailability permeability in the caco-2 cell model.[3] The permeability enhancers have the limitations of causing irritation of the epithelial layer and, moreover, lots of these enhancers have not been approved by the US Food and Drug Administration.

With respect to the solubility improvement of RBM, rebamipide lysinate has been used to determine the effects of solubility enhancement on the bioavailability of the drug in a rat model.[4] Although repamipide lysinate improved the solubility of RBM in water 17 times, this salt also offered the same area under the curve (AUC) to that of rebamipide. However, these studies have not shown any impact of bioavailability enhancement (higher permeability or solubility) on the pharmacological activity of rebamipide.

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Besides the application of the salt form to increase the solubility of a drug,[4] other methods have also been suggested.[5–7] Using co-solvents to increase the solubility of drugs is a traditional method for solubility enhancement of poorly soluble drugs.[7] However, drugs are always faced with a precipitation problem when used in a cosolvent system. In an attempt to overcome this shortcoming, newer methods in which drugs are incorporated with at least one kind of hydrophilic polymer or polymeric surfactant have been developed. Inclusion complexes of nicardipine and hyroxypropyl cyclodextrin have increased the solubility of these drugs above 90%.[5] The absorption of simvastatin acid from self-microemulsifying systems (SMEDDS) was about 1.5-fold higher in bioavailability than the conventional tablet. Recently, nanosuspension has emerged as a potential technique to increase the solubility of poorly soluble drugs.[8] However, the question is then: what is the appropriate method for a particular drug like rebamipide?

Consequently, the first purpose of this study was to develop an appropriate method for rebamipide solubility enhancement. Moreover, the study also determined the effect of solubility enhancement on the bioavailability and efficacy of rebamipide.

Materials and Methods

Materials

RBM was obtained from Jin Yang Co. Ltd (Korea). Lutrol® F 127 (poloxamer 407) and Kollidone® VA 64 (PVP-VA 64) were purchased from BASF Co., Ltd (Germany). L-lysine was purchased from TCI Co. Ltd (Japan). Water was purified by reverse osmosis and filtered in house. The original and non-specific tablet containing rebamipide (Mucosta® 100 mg) was purchased from Osuka Co. Ltd (Japan) as the reference product. All other reagents were analytical grade commercial products.

Animals

Thirty-one male white Sprague–Dawley rats were obtained from Samtako Co. Ltd (Korea), each having a weight between 200 and 280 g. These were used in the conduct of the pharmacokinetic (PK) and pharmacodynamic (PD) test. They were kept in a clean room at a temperature of 23 ± 2°C with a 12-h light/dark cycle. The relative humidity was 55 ± 15% with air ventilation frequency of 15–20 times/h. All rats were fed with water and commercial diet. The protocol of the animal study was approved by the Animal Care and Use Committee of the School of Pharmacy, Sungkyunkwan University.

Determination of solubility of rebamipide

An excess amount of RBM was weighed and added to 5 ml of the medium in a screw-capped glass test-tube. The suspension was shaken at 100 rpm in a thermostatic water bath (model SWB-03, Jeio Tech Co., Korea) at 25 ± 0.5°C. After 48 h, the suspension was centrifuged at 4000 rpm for 10 min and filtered with a membrane filter (nylon, 0.45 μm, Whatman®, UK) to remove the undissolved substance. The clear solution was diluted adequately with the mobile phase and analysed using a validated HPLC method.

Preparation of solid dispersion

According to a previous report,[4] the solubility of RBM strongly depends on the pH of the dissolution medium and rebamipide lysinate improves the solubility of RBM in water by 17 times. The primary solid dispersion (SD) was therefore prepared from a solution containing rebamipide, L-lysine and PVP-VA 64 in water and ethanol. The primary solid dispersion was prepared in an Eyela Spray Dryer (model SD-1, Tokyo Rikakikai Co., Ltd, Japan) with inlet and outlet temperatures of about 100 and 70°C, respectively. Air pressure and pump rate were about 1.5 bar and 5 ml/min, respectively.

To further improve the rate and extent of surface wetting ability of the spray-dried powder in pH 1.2, the primary solid dispersion was ground with different levels of poloxamer 407 using a pestle and mortar. These mixtures were passed through a #80 mesh sieve to obtain the final solid dispersion mixtures. To compare the effect of the solid dispersion mixtures with the original product (Mucosta®), the dissolution rate of RBM from the solid dispersion and Mucosta® tablets was measured at a pH of 1.2. Due to the fast disintegration of Mucosta® tablet (1–2 min), it was not necessary to grind or otherwise modify the steps for the reference tablets.

In-vitro release study from solid dispersion

Dissolution test of rebamipide

Dissolution of RBM from the solid dispersion powder and Mucosta® tablets were studied using the KP dissolution apparatus type 2 (model DST-810, Labfine, Korea). The dissolution media were 900 ml of 0.1 M pH 1.2 (7 ml HCl and 2 g NaCl per 1000 ml distilled water) or 0.05 M pH 6.8 buffer (6.805 g KH_2PO_4 and 0.944 g NaOH per 1000 ml distilled water). The temperature was maintained at 37 ± 0.5°C. The rotation speed was 100 rpm. Five milliliters of aliquot were withdrawn at predetermined time intervals of 30, 60 and 120 min and filtered through membranes (nylon, 0.45 μm, Whatman®, UK). The medium was replenished with 5 ml of fresh buffer each time. Samples were analysed using an HPLC system.

Analysis of rebamipide in dissolution medium using HPLC system

The withdrawn samples were analysed using an HPLC system (Hitachi, Japan) consisting of an isocratic pump (model L-7100), an automatic injector (model L-7200), an integrator (model L-7000) and a UV detector (model L-7400). The detector was set at 327 nm. The analytical column was Luna (150 × 4.6 mm ID, Phenomenex, USA). The mobile phase of acetate buffer : methanol : glacial acid acetic (510 : 490 : 20) was delivered at 1 ml/min. The volume injection was 20 μl. The temperature was set at 25°C. The stock solution was prepared by solubilizing RBM in methanol. The range of standard samples was from 5 μg/ml to 100 μg/ml. Validation of HPLC methods was conducted by calculating the percentage deviation and the percentage coefficient of variation from three calibration curves.

Dissolution data treatment

Dissolution profiles of each formula were compared by determining kinetic models (zeroth, first, second order, Higuchi, Hixon–Crowell, Korsmeyer–Peppas, Weibull). After primary data analysis, the simple and semi-empirical model, Korsmeyer–Peppas, was used for comparison because it had the highest coefficient:

$$\frac{M_t}{M_\infty} = Kt^N$$

where M_t is the amount of RBM released at time t, M_∞ is the amount of drug released at time infinity (%), N is the release exponent and K is the release constant.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using Perkin Elmer Pyris DSC thermal analyser system in aluminium pans. DSC was performed against a reference pan at temperatures between 40 and 350°C at 10°C/min.

Fourier transform infrared spectroscopy

FT-IR spectra were obtained on a Fourier transform infrared spectroscope (Model IFS-66/S, Bruker Optics, Germany) using the potassium bromide (KBr) disk method. One to two milligrams of sample was mixed with 150 mg of spectra-grade KBr and pressed into a disk of 12-mm diameter using a Carver hydraulic press (model 3912, Carver Inc., USA). Samples were analysed from 4000 to 600 cm^{-1} with an instrument resolution of 0.1 cm^{-1}.

In-vivo evaluation of solid dispersion

Sample preparation for in-vivo studies

Test samples were prepared by suspending the solid dispersion mixtures of different dosages of RBM in a solution of sodium carboxymethyl cellulose 0.5%. Meanwhile, reference samples were prepared by grinding Mucosta® tablets with a pestle and mortar. The ground powders were sieved through a #80 mesh sieve to obtain fine powders having the same size as the solid dispersion mixture. These ground powders, with different dosages of RBM, were also suspended in sodium carboxymethyl cellulose 0.5% to be ready for the PK and PD studies.

Pharmacokinetic studies

Fifteen rats were cannulated with polyethylene tubes (SP 45, ID 0.58 mm, OD 0.96 mm) and silastic tubes (ID 0.025 in, OD 0.047 in) into the jugular vein and kept in the fasting condition for one night before the day of the experiment. Rats were administered with solid dispersion and reference suspensions containing the dosage of RBM: 5 and 10 mg/kg. Five rats were used for each formulation. Blood samples (about 500 µl) were withdrawn from the jugular vein after 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h, and were supplemented with equal amounts of saline containing heparin 50 UI. Plasma was collected by centrifugation of the above blood at 10000 rpm within 10 min and kept in a deep-freezer at –40°C until the day of analysis.

Pharmacodynamic studies

The local effects of rebampide were determined by inducing rat stomach ulcers with a suspension of ketoprofen 5 mg/kg in NaCMC 0.5% and ethanol 100%. Firstly, 16 rats were administered suspensions of control reference (50 and 100 mg/kg) and solid dispersion (50 mg/kg) in NaCMC 0.5% to simulate protective factors in the stomach: increasing cycloxygenase, growth factors, prostaglandin E receptors, decreasing neutrophil activity and inflammation process.[1] Four rats were used for each formulation. Stomach irritation was induced continuously by ketoprofen (5 mg/kg) and ethanol 100% (4 ml/kg after 0.5 and 2.5 h). Rats were sacrificed after 12 h. The abdominal cavities of the rats were incised, and the stomach tissues were removed and fixed with 10 ml of 2% formalin for 60 min. Irritation areas of stomach were determined by capturing a picture of stomach on scaled papers and calculating the irritation area by Image-Pro Express software (Media Cybernetics, USA). The percentage inhibition of stomach irritation was calculated by the following equation:

$$\%\text{inhibition} = \frac{\text{control irritation area } (mm^2) - \text{drug treated irritation area } (mm^2)}{\text{control irritaion area } (mm^2)} \times 100$$

Analysis of rebamipide in rat plasma using HPLC

RBM in plasma was analysed with an HPLC system (Hitachi, Japan) consisting of an isocratic pump (model L-7100), an automatic injector (model L-7200), an integrator (model L-7000) and a fluorescence detector (model FL-2480). The excitation and emission wavelengths were 331 and 379 nm, respectively. The analytical column was Luna (150 × 4.6 mm ID, Phenomenex, USA) and precolumn (30 × 4.6 mm ID). The mobile phase, containing methanol : pH 2.5 phosphate buffer 0.05 M (50 : 50 V/V) and 0.2% triethylamine (adjusted to pH 2.5 with phosphoric acid 85%), was delivered at 1 ml/min. The volume of injection was 20 µl. The temperature was set at 50°C.[9]

Plasma was prepared by adding 100µl plasma, 10 µl H_3PO_4 50% and 10 µl ofloxacin 50 µg/ml into a glass tube and vortexing all components for 5 s. RBMwas extracted by adding 1 ml ethyl acetate, vortexing for 5 min and centrifuging at 3500 rpm for 10 min. The supernatant was transferred to a 5-ml glass tube and evaporating under N_2 at 55°C. The residue was reconstituted with 100µl mobile phase, injected to the vial, and was then ready for analysis by HPLC.[9] The range of standard samples was from 0.01 to 1 µg/ml. Validation of HPLC methods was conducted by calculating percentage deviation and the percentage coefficient of variation from three calibration curves.

Data analysis

The data were calculated using Excel (Microsoft, USA) and WinNonlin (Scientific Consulting Inc., USA) programs. Data were expressed as mean ± SD and analysed for statistical significance by one-way ANOVA and Student's *t*-test using Excel (Microsoft 2007, USA).

Table 1 The precision and accuracy evaluation for the rebamipide assay in dissolution test by HPLC-UV detector and in rat plasma by HPLC-fluorescent detector

HPLC-UV			HPLC-FL		
Concentration (μg/ml)	Average (% deviation)	% C.V	Concentration (μg/ml)	Average (% deviation)	% C.V
			0.01	18.20	18.00
5.00	4.20	5.24	0.05	7.80	10.64
10.00	6.51	1.83	0.1	10.19	3.06
50.00	3.06	4.08	0.5	1.86	2.40
100.00	0.79	1.00	1	3.88	2.42
Mean	3.64	3.04	Mean	8.39	7.31

C.V, coefficient of variation.

Table 2 Solubility values of RBM in some of the commonly used solvents ($n = 3$, mean ± SD)

Solvents	Concentration (mg/ml)
N-2-methyl pyrrolidone	282.26 ± 1.50
Dimethyl sulfoxide	51.92 ± 0.28
PEG 200	2.95 ± 0.03
PEG 400	1.94 ± 0.22
Tween 80	1.93 ± 0.04
PEG 300	1.89 ± 0.19
Polyethylene glycol	1.39 ± 0.06
Transcutol	1.34 ± 0.05
Pluronic L-10	0.67 ± 0.03
Labrasol	0.56 ± 0.01
Ethanol	0.56 ± 0.02
Capryol 90	0.40 ± 0.02
Labrafac hydrophilic	<0.01
Glycerin	<0.01
Lauryglycol	<0.01
Triacetin	<0.01
Isopropyl alcohol mirystate	<0.01
Miglyol 812	<0.01
Dicholoromethane	<0.01

Results

The precision and accuracy evaluation for the rebamipide assay in the dissolution test by HPLC-UV and in rat plasma by HPLC-fluorescent detector are shown in Table 1. The mean percentage deviation and percentage coefficient of variation of the concentrations of RBM determined by HPLC-UV were 3.64 and 3.04%, respectively. Additionally, mean percentage deviation and the percentage coefficient of variation of RBM concentrations determined by HPLC-fluorescence were 8.39 and 7.31%, respectively. The individual values of each standard sample were lower than 20% (Table 1). Moreover, the correlation coefficients of all calibration curves equaled 0.9999. These data mean the linear concentration range of RBM is from 5 to 100 μg/ml and from 0.01 to 1 μg/ml with HPLC-UV and HPLC-fluorescence, respectively.

According to the solubility test and the United States Pharmacopeia (USP) standard, RBM was almost insoluble (<1 g/1000 ml) both in polar and non-polar solvents (Table 2). This drug is only freely soluble in N-2 methyl pyrrolidone (282.26 ± 1.50 mg/ml) and less soluble in dimethyl sulfoxide (51.92 ± 0.28 mg/ml).

To determine the effects of poloxamer 407 on the dissolution rates of RBM at a pH of 1.2, the compositions of spray-dried powder were maintained at a ratio of 100 mg RBM to 400 mg PVP-VA 64, and the amount of poloxamer 407 was increased from 0 to 800 mg (F1–F5) (Table 3). The dissolution rate of RBM from each formulation was proportional to the release constant, K, which is shown in Table 4. Generally, the K value increased proportionally to the amount of poloxamer (from F1 at 0.1178 up to F4 at 0.4613). Nevertheless, when using 800 mg poloxamer in F5, the K constant reduced to 0.2870. Among the five formulations, F3 and F4 had the highest K values, at 0.4182 and 0.4613, respectively. However, analysis of variance showed that at 2 h F3, using 200 mg of poloxamer 407, had a higher dissolution rate of RBM than F4 (34.96 vs 30.47%; $P < 0.05$). Therefore, the ratio of 200 mg poloxamer 407 to 100 mg RBM was fixed for further studies (Figure 1).

The effect of PVP-VA 64 on the dissolution rate of RBM in pH 1.2 was studied by adjusting the amount of PVP-VA 64 in F3 from 0 to 400 mg (F6, F7, F8, F9, F3) (Table 3). The K values of F6 (0 mg PVP-VA 64) and F7 (50 mg PVP-VA 64) were 0.6790 and 0.5419, respectively. This release constant of RBM significantly increased to the highest level in F8 (100 mg PVP-VA 64) at 0.7427. Meanwhile, F9 and F3, using higher levels of PVP-VA 64 (200 and 400 mg), had lower release constants of 0.4651 and 0.4182 (Table 4). After 2 h, F8 showed the highest dissolution rate of RBM at 62.17% ($P < 0.05$) (Figure 2). F8 was chosen for the PK and PD tests.

To further confirm the effect of solid dispersion along the whole length of the gastrointestinal medium, a dissolution test of F8 powder and a reference product was conducted at pH 1.2 and pH 6.8. Mucosta® tablets were chosen as the reference product for two reasons. Firstly, Mucosta® is an original product and widely marketed. Secondly, Mucosta® is a non-specific tablet and disintegrates quickly to form a powder or granule state when kept in dissolution conditions. Consequently, Mucosta® was still in a powder state after 1–2 min in the pH-1.2, 900-rpm dissolution test, a treatment that was adequate to dissolve F8. After 2 h, the release rate of RBM from F8 at pH 1.2 was 62.17% higher than that of Mucosta® tablets. Meanwhile, both formula F8 and the Mucosta® tablet had a 100% release of RBM at pH 6.8 (Figure 3).

The mechanism of solubility enhancement was explained by DSC and FT-IR. According to DSC, RBM has very high melting point (306°C). This peak disappeared in the physical mixture, the primary solid dispersion and F8 (Figure 4).

Table 3 Formulation of RBM with various amounts of poloxamer 407 and PVP-VA 64

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Rebamipide (mg)	100	100	100	100	100	100	100	100	100
L-lysine (mg)	80	80	80	80	80	80	80	80	80
PVP-VA 64 (mg)	400	400	400	400	400	0	50	100	200
Poloxamer 407 (mg)	0	100	200	400	800	200	200	200	200
Aerosil (mg)	20	20	20	20	20	20	20	20	20
Water (mL)	8	8	8	8	8	8	8	8	8
Ethanol (mL)	8	8	8	8	8	8	8	8	8

Table 4 Release constant, release exponent and efficient correlation of RBM from solid dispersion formula and Mucosta® tablet in pH 1.2

	K	N	R^2
F1	0.1178	0.9049	0.9954
F2	0.3262	0.9516	0.9998
F3	0.4182	0.9620	0.9998
F4	0.4613	0.9654	0.9992
F5	0.2870	0.9454	0.9998
F6	0.6790	0.9823	0.9994
F7	0.5419	0.9730	0.9998
F8	0.7427	0.9866	0.9997
F9	0.4651	0.9667	0.9999
Mucosta	0.0000	0.0000	0.0000

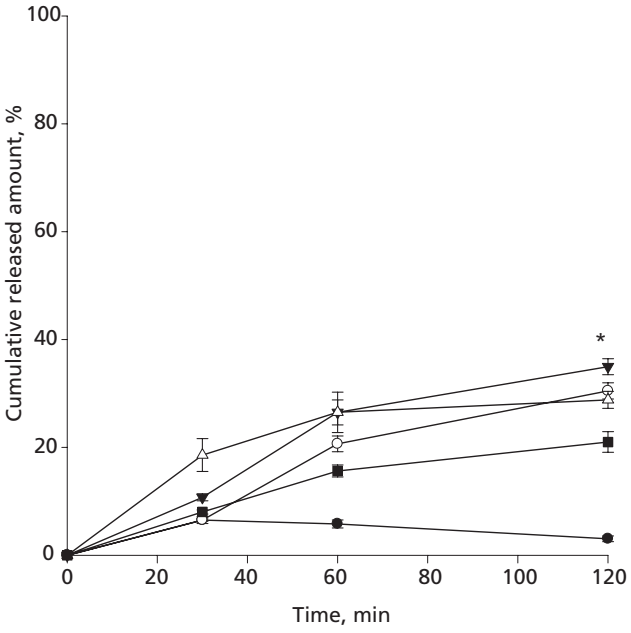

Figure 1 Dissolution profiles of RBM from solid dispersions at pH 1.2 containing PVP-VA 64:poloxamer 407:RBM, with different amounts of poloxamer 407. ●, F1, 0 mg poloxamer 407; o, F2, 100 mg poloxamer 407; ▼, F3, 200 mg poloxamer 407; △, F4, 400 mg poloxamer 407; ■, F5, 800 mg poloxamer 407. Values shown for $n = 3$, mean ± SD, $^*P < 0.05$.

With regard to the FT-IR result, RBM had a specific peak for a carbonyl group at 1643 cm^{-1}. This peak was still presented in the physical mixture and only moved to 1661 cm^{-1} in the primary solid dispersion and F8. PVP-VA 64 had its

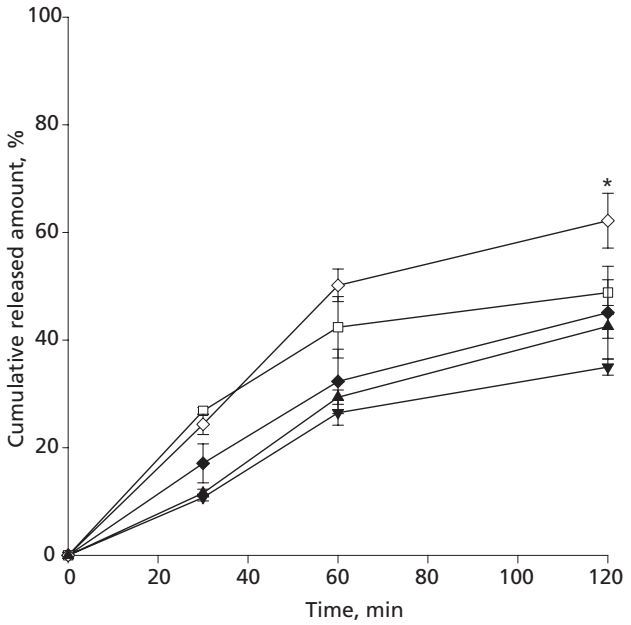

Figure 2 Dissolution profiles of RBM from solid dispersions at pH 1.2 containing PVP-VA 64:poloxamer 407:RBM with different amounts of PVP-VA 64. □, F6, 0 mg PVP-VA 64; ◆, F7, 50 mg PVP-VA 64; ◇, F8, 100 mg PVP-VA 64; ▲, F9, 200 mg PVP-VA 64; ▼, F3, 400 mg PVP-VA 64. Values shown for $n = 3$, mean ± SD, $^*P < 0.05$.

carbonyl peak at 1673 cm^{-1} but this was hidden by the carbonyl group of RBM at 1643 cm^{-1} in the physical mixture. It was slightly shifted to 1692 cm^{-1} in the primary solid dispersion and F8 (Figure 5).

F8 was used to carry out the PK study in a rat model with the dosage of RBM set at 5 and 10 mg/kg. The study was conducted simultaneously with a Mucosta® suspension (10 mg/kg of RBM; Figure 6). After 24 h of administration, the AUC of RBM from F8 at 10 mg/kg and the Mucosta® 10 mg/kg suspension were 847.85 ± 176.51 and 486.93 ± 176.51 ng.h/ml, respectively (Table 5). The bioavailability of RBM from F8 was 1.74 times higher than that of the Mucosta® suspension.

The PD study of RBM compared the effect of F8 (50 mg/kg) with Mucosta® suspension (50 and 100 mg/kg). Under strong irritation conditions – ketoprofen 5 mg/kg and ethanol 100% – the control samples showed a severe irritation area of 188.70 ± 53.16 mm^2. However, when the rats were treated with reference (50 and 100 mg/kg) or F8 (50 mg/kg), the irritation areas were reduced significantly

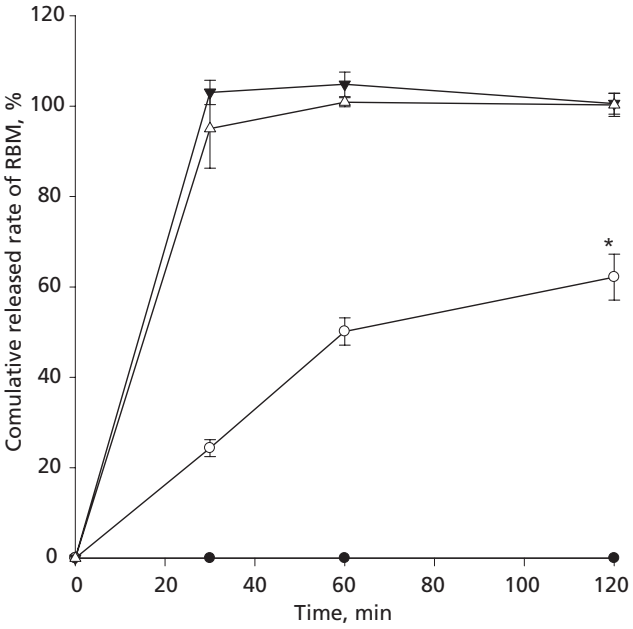

Figure 3 Dissolution profiles of RBM from: ●, Mucosta® (100 mg) tablet at pH 1.2; o, F8 at pH 1.2; ▼, Mucosta® (100 mg) tablet at pH 6.8; △, F8 at pH 6.8. Values shown for $n = 3$, mean ± SD, $*P < 0.05$.

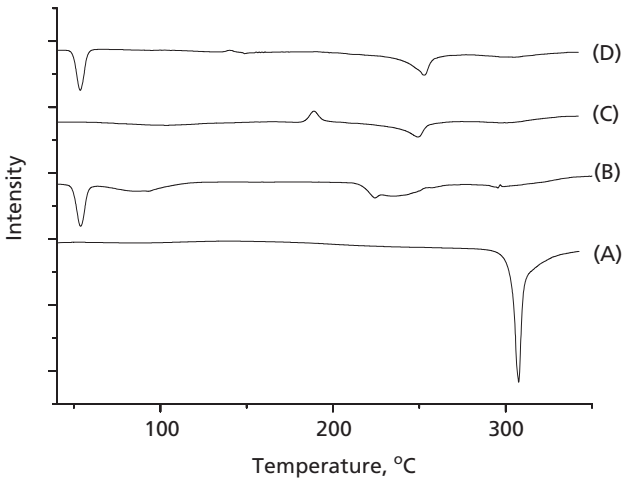

Figure 4 DSC thermograms of samples; A, rebamipide; B, physical mixture; C, primary solid disopersion; D, solid dispersion F.

(Figure 7). The percentage inhibition rates of Mucosta® (50 and 100 mg/kg) and F8 (50 mg/kg) were 27.92, 42.65 and 75.70%, respectively.

Discussion

Our strategy to increase the solubility of RBM was based on some fundamental factors. Firstly, according to USP standards for solubility levels of a drug, RBM was insoluble (<1 g/1000 ml) or very slightly soluble (1 g/300 ml–1 g/1000 ml) in polar solvents such as ethanol, dicholomethane, propylene glycol and glycerin, and insoluble in non-polar solvents such as isopropyl myristate and miglyol 812 (Table 2), therefore it

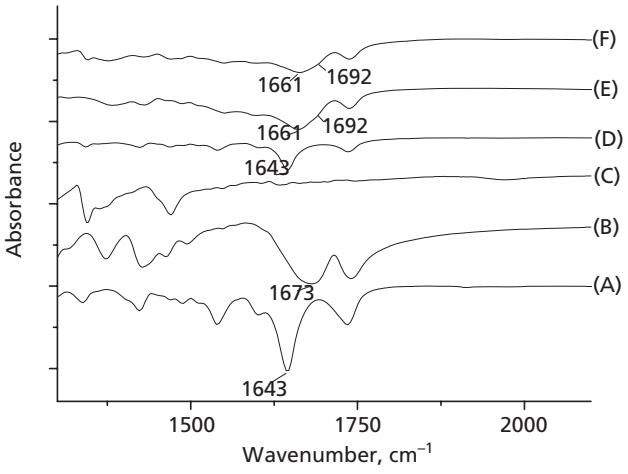

Figure 5 FT-IR spectra of carbonyl stretching region comparing solid dispersions of rebamipide, poloxamer 407 and PVP-VA 64 with physical mixtures of corresponding composition. A, rebamipide; B, PVP-VA 64; C, poloxamer 407; D, physical mixture; E, primary solid dispersion; F, solid dispersion F8.

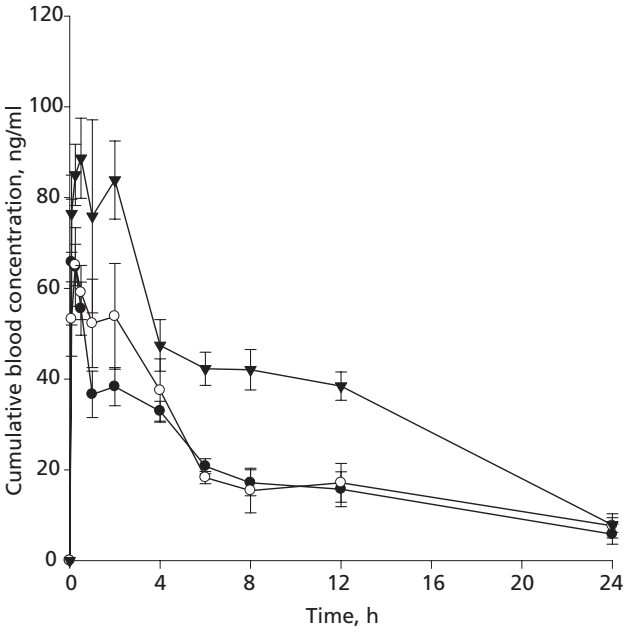

Figure 6 Pharmacokinetic profiles of RBM in rat model from: ●, F8 with 5 mg/kg of RBM; ○, Mucosta® suspension with 10 mg/kg of RBM; ▼, F8 with 10 mg/kg of RBM. Values shown for $n = 5$, mean ± SE.

is hard to prepare a solid dispersion by the direct evaporation method or SMEDDS. Secondly, because RBM has a very high melting point (306°C), it is also not practical to prepare a solid dispersion by the hot-melt method. Thirdly, a high dose of RBM (100 mg per tablet) also restricts the application of nanosuspensions and micellar dispersions to solubility enhancement.[6] Fortunately, since RBM has a polar carboxyl group, pH adjustments or suitable salt formation is one strategy to increase its solubility. According to Beom, [4] even though rebamipide lysinate improves the solubility of RBM

Table 5 Pharmacokinetic parameters of RBM from reference product Mucosta® suspension with 10 mg/kg of RBM, and from solid dispersion (F8) solution with 5 and 10 mg/kg of RBM ($n = 5$, mean ± SD)

Parameters	RBM (10 mg/kg)	F8 (10 mg/kg)	F8 (5 mg/kg)
AUC (ng.h/ml)	486.93 ± 176.51	847.85 ± 122.08*	422.03 ± 83.52
T_{max} (h)	0.75 ± 0.77	1.38 ± 0.75	0.23 ± 0.17
C_{max} (ng/ml)	73.00 ± 9.99	101.04 ± 25.44	71.91 ± 29.18
$t_{1/2}$ (h)	6.90 ± 0.56	6.74 ± 0.72	5.29 ± 0.80
V_z/F (L/kg)	0.19 ± 0.07	0.11 ± 0.02	0.09 ± 0.02
CL/F (ml/min/kg)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
MRT (h)	6.81 ± 2.02	8.15 ± 0.28	7.69 ± 1.00

*$P < 0.05$, comparison AUC of F-8 with Mucosta® by Student's t-test.

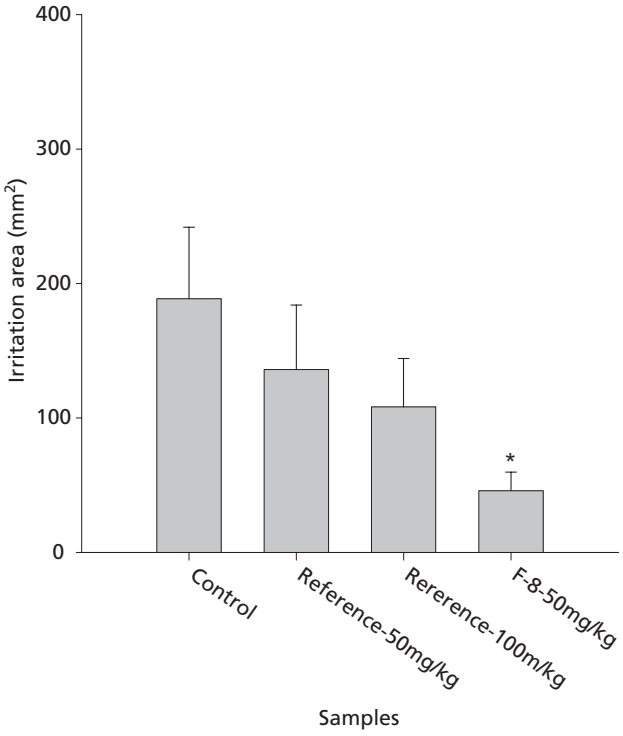

Figure 7 Irritation area of rat stomach, following treatment with reference Mucosta® suspension (50 and 100 mg/kg) and F8 (50 mg/kg). *$P < 0.05$, comparison of F8 (50 mg/kg) with control, reference (50 and 100 mg/kg) by ANOVA test. Values shown for $n = 4$, mean ± SE.

by 17 times in water, this weak salt is not useful in further increasing bioavailability. Therefore, a solid dispersion containing RBM, lysine, PVP-VA 64 and poloxamer 407 was developed.

Because the maximum retention time of the drug in the stomach medium was around 2 h and RBM is insoluble at pH 1.2, the impact of solubility enhancement from different formulations was compared by measuring the dissolution rate of RBM after 2 h. Furthermore, the release rate of RBM from dissolution points before 2 h were always lower than the equivalent values at 2 h. Therefore the number of dissolution points before 2 h were minimized to save time and labour.

Poloxamer 407 is known as a non-ionic triblock surfactant consisting of two hydrophilic segments and one hydrophobic segment.[10] At low concentrations, this surfactant exists as a monomolecule and decreases surface tension as well as the surface free energy. The release constants of RBM from F1 and F2 were 0.1178 and 0.3262, respectively. However, with an increase in the concentration of poloxamer, multimolecular aggregation occurs.[10] Under the interaction of Van der Waals forces between polypropylene oxide (PPO) and a substance, as well as the hydrophobic interaction of PPO segments, a central hydrophobic core forms to contain the drug. Meanwhile, polyethylene oxide (PEO) segments form a corona, which is responsible for hydrogen bonding of its ether oxygen with water molecules, thereby increasing the solubility of drug. Because of the high ratio of both the PPO portion and the PEO portions, (101 and 56 respectively), micelles of poloxamer 407 are reported to be highly useful in increasing the solubility of drugs.[10] As a result, the release constants of F3 and F4 were significantly better than those of F1 and F2. However, there is a critical micelle concentration, and when an excess amount of polxamer 407 was used the micelles packed into a cubic structure in the form of a gel. Only 28.8 and 20.9% RBM was released from F4 and F5 partly because of the strong complex of RBM formed in this core of dense micelles and partly because of the low diffusion rate of RBM in the high-viscosity medium.

PVP-VA 64, a fast dissolving polymer, was used in some previously published papers on binary solid dispersions containing PVP-VA 64 with another polymer.[11–13] Accordingly, this fast-dissolving polymer has played the role of a carrier to maintain the molecular dispersion of the drug in solid dispersions.[12] Other polymers combined with PVP-VA 64 are slow-dissolving polymers such as Eudrgit E100[12] or surfactants such as TPGS 1000 and Myrj 52.[11,13] The purpose of combining with these polymers is to improve the solubility of the drug. The results of IR and FT-Raman spectra show the interaction of indomethacin with PVP and PVP-VA 64.[14,15] Hydrogen bonds form between the carboxylic acid dimers of indomathacin and the polymer carbonyls, resulting in disruption of the indomethacin dimers. The crystallization kinetics of indomethacin are influenced by the fact that PVP prevents the self-association of indomethacin molecules. Both RBM and indomethacin are weak acid agents containing a similar functional group, the carboxylic acid. We therefore applied PVP-VA 64 in the case of RBM. When the amount of PVP-VA 64 was at a low level, the polymer was not effective in inhibiting crystallization, and we obtained only 48.8 and 45.1% release of RBM from F6 and F7. Meanwhile, an excess amount of PVP-VA 64 (F3, F9) caused high viscosity in the

medium, thereby inhibiting the diffusion of RBM from the surface layer of the drug particle (Figure 2).

Figure 3 shows that the solubility of RBM is strongly dependent on pH. Under the effect of the binary solid dispersion, RBM from F8 was maintained at a high concentration in both gastric and intestinal media. Accordingly, F8 gives RBM the ability to maintain contact and penetrate the absorption surface at a molecular level. This finding was further confirmed in the PK study.

The peak at 306°C in the DSC analysis represents the crystallized state of RBM. The disappearance of this peak in the primary solid dispersion and F8 illustrates the amorphous state of RBM in the solid dispersion system, explaining the significant solubility enhancement of RBM. In the case of the physical mixture, because of the early melting phenomenon of poloxamer at 53.6°C, the crystal peak of RBM at 306°C is hidden and inhibited in the DSC.

The movement of the carbonyl group of RBM from 1643 to 1661 cm^{-1} means that the carboxylic acid group of RBM is affected by a new bond. Moreover, the shifting of the specific peak (–C=O) of PVP-VA 64 from 1673 to 1692 cm^{-1} indicates that this proton-donor group interacts with a proton-receiving group of RBM (–OH in the carboxylic acid group of RBM). These hydrogen bonds cause the change in position of the –C=O peak of RBM. Consequently, hydrogen bonds forming between the carboxylic acid of RBM and PVP-VA 64 carbonyl result in disruption of the crystal structure of RBM.

The solid dispersion increases the bioavailability of the drug in the biopharmaceutics classification system class IV (Table 5). The AUCs of RBM from F8 (10 mg/kg) and reference (10 mg/kg) were 847.85 ± 122.08 and 486.93 ± 176.51 ng.h/ml, respectively. This phenomenon was explained by the fact that the high solubility of RBM along the whole length of the gastrointestinal tract enables RBM to get sufficient contact with the absorption epithelium of oral route. Wong has reported that the particles containing poloxamer 407 have high wetting ability for drug particles.[16] This phenomenon prevents the aggregation of particles when exposed to the aqueous medium of the gastrointestinal fluid. Consequently, particles present a larger specific surface area for dissolution. In addition, in the gastrointestinal fluid poloxamer molecules also form micelles to contain RBM. The nanoscale size of these micelles generally creates a high ability to deliver RBM to the blood by the paracellular pathway.

RBM is useful when administered before induction of ulcers by irritant agents, simulating mucosal protection factors in the stomach: increasing cycloxygenase, growth factors, prostaglandin E receptors and decreasing neutrophils.[17,18] The PK studies show that the AUC of RBM from F8 (10 mg/kg) was 1.74 times higher than that of reference (10 mg/kg) after 12 h. As a result, the PD study was conducted in a long-term experiment of about 12 h. The data obtained show that the irritation area of the stomach depended on the dosage of RBM, the solubility of RBM at a pH of 1.2 and the bioavailability of drug in the blood plasma. Based on the relationship of bioavailability, solubility and drug efficacy, there were two factors explaining the 2.71-times reduction of the irritation area. Firstly, bioavailability enhancement (1.74 times) increased the presentation of RBM in the blood circulation, thus promoting the ulcer healing process in the stomach via a systemic pathway. Secondly, the high solubility of RBM (62.17%) from F8 in the stomach increases the local concentration and local penetration of RBM in the gastric mucosa and gastric mucus. As a result, there is an increase in the generation of endogenous prostaglandins in the gastric mucosa, which plays a pivotal role in maintaining the mucosal-protective and antisecretory effects of RBM.[17]

Conclusions

This study made use of some physiochemical parameters to decide the strategy for solubility enhancement of a low-aqueous-soluble drug. The solid dispersion system proved its usefulness. It combined an alkaline agent (or acidic agent) with a polymer and surfactant to increase the solubility of a drug of pH-dependent solubility. Even though the study did not show the minimum limit of solubility enhancement with RBM to cause a clinical response, the results illustrate the proportional impact of solubility improvement on bioavailability and efficacy of RBM.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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