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Preparation and *in vitro*/*in vivo* evaluation of revaprazan hydrochloride nanosuspension

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ABSTRACT

Revaprazan hydrochloride (RH) is a new reversible proton pump inhibitor. However, due to poor water solubility, oral bioavailability of the drug was relatively low. To investigate the particle size reduction effect of RH on dissolution and absorption, three suspensions that containing different sized particles were prepared by high pressure homogenization and *in vitro*/*in vivo* evaluations were carried out. DSC and powder X-ray diffraction were used to study crystalline state of freeze dried powder of RH suspensions and the results showed that particles of RH microsuspension and nanosuspension remained in the same crystalline state as coarse suspension, but had lower lattice energy. In the *in vitro* dissolution test, both microsuspension and nanosuspension showed increased dissolution rate. In the *in vivo* evaluation, compared to coarse suspension, RH nanosuspension exhibited significant increase in AUC_{0-t}, C_{max} and decrease in T_{max}, MRT. Nevertheless, RH microsuspension did not display any significant differences in these pharmacokinetic parameters compared to the coarse suspension. The findings revealed that particle size reduction can influence RH absorption in gastrointestinal tract and nanosuspension can enhance oral bioavailability of RH in rats.

1. Introduction

Revaprazan hydrochloride (RH) [5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl) pyrimidine hydrochlorate, Fig. 1], is a new reversible proton pump inhibitor. In contrast to irreversible proton pump inhibitors, such as omeprazole and lansoprazole, RH reversibly inhibits H^+/K^+-ATPase via binding to the K^+-binding site of the pump. This reversibility leads to fewer adverse events (Hwang et al., 1998). Nevertheless, pharmacokinetic studies of RH in rats and dogs showed the drug had a low oral bioavailability, which was speculated to be due to first pass effect and poor water solubility of drug. Since the percentage of unchanged drug remaining in the gastrointestinal tract (caused by poor water solubility, 0.11 mg/ml) increased with dose (Han et al., 1998), improving dissolution rate of drug becomes the rational approach to overcome this problem. There are many classical pharmaceutical ways to improve drug dissolution rate such as dissolution in aqueous mixtures with an organic solvent (Stovall et al., 2005), formation of β-cyclodextrin complexes (Loftsson and Brewster, 1996; Loftsson et al., 2004; Wang et al., 2006; Makhlof et al., 2008), solid dispersions (Park and Hyun, 2008) and drug salt form (Tao et al., 2009).

During the past 20 years a new technology, reducing drug particle size, has been developed to increase drug dissolution rate. According to Noyes–Whitney equation, drugs with smaller particle size have enlarged surface areas which lead to increased dissolution velocity (Noyes and Whitney, 1897). And higher dissolution rate together with the resulting higher concentration gradient between gastrointestinal lumen and blood could further increase oral bioavailability of drugs (Liversidge and Conzentino, 1995; Jinno et al., 2006). There are various ways to produce drug nanosuspensions or nanoparticles, from top down processes and bottom up processes to the combination of the two methods. Among these technologies high pressure homogenization (HPH) is widely used because of its simplicity of the process, ease of large scale production, absence of organic solvents and reduced product contamination (Müller and Bohn, 1998; Müller and Akkar, 2004).

The aim of the present study was to prepare RH nanosuspension by high pressure homogenization and investigate the influence of particle size reduction on drug dissolution rate as well as oral bioavailability of RH nanosuspension in rats.

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2. Materials and methods

2.1. Materials

Revaprazan hydrochloride (the purity of revaprazan hydrochloride is up to 98%; ^{1}H NMR (300 MHz, $CDCl_3$, ppm), δ: 1.61–1.64 (3H, m), 2.20 (3H, S) 2.45 (3H, S), 2.84–2.90 (1H, m), 3.12–3.23 (1H, m), 3.54–3.64 (1H, m), 4.24–4.30 (1H, m), 5.34–5.41 (1H, m), 7.02–7.10 (3H, m), 7.13–7.18 (1H, m), 7.20–7.24 (2H, m), 7.50–7.55 (2H, m), 10.21 (1H, S), 14.03 (1H, S); ^{13}C NMR (300 MHz, $CDCl_3$, ppm), δ: 14.76, 18.20, 21.71, 28.45, 41.03, 54.44, 103.21, 115.29, 115.59, 123.16, 123.26, 126.61, 126.73, 127.08, 129.12, 132.37, 132.86, 137.48, 150.11, 152.88, 157.68, 164.85; ESI-MS m/z: 363.05 [M^+]) was synthesized in the laboratory of Dr. Tian (Shenyang Pharmaceutical University, China). Poloxamer 188 was kindly gifted by BASF (Ludwigshafen, Germany). Methanol and acetonitrile of HPLC grade were purchased from Jiangsu Hanbon Science & Technology Co., Ltd. (Jiangsu, China). All other reagents were analytical grade commercial products.

2.2. Preparation of RH/poloxamer 188 physical mixture

The physical mixture was prepared by blending RH powder and poloxamer 188 powder in a mortar until a homogenous mixture was obtained. The ratio of RH powder/poloxamer 188 powder was 2:1 (w/w).

2.3. Preparation of RH coarse suspension

RH coarse powder was grinded in a mortar for 10 min and then dispersed in 0.5% (w/w) poloxamer 188 bidistilled water solutions. Then the obtained suspension was placed in an ultrasonic water bath (Shenyang Ultrasonic Technology Co. Ltd., Shenyang, China) for 20 min and finally RH coarse suspension was prepared. Coarse suspension was prepared using 1:100 drug/water ratio (w/w).

2.4. Preparation of RH microsuspension

The grinded coarse RH powder was dispersed in poloxamer 188 bidistilled water solution and then placed in ultrasonic water bath (Shenyang Ultrasonic Technology Co. Ltd., Shenyang, China) for 20 min. The obtained suspension was homogenized at high pressure (2 cycles at 90 bar, 15 cycles at 150 bar) by high pressure homogenization using an AH 100 apparatus (ATS Engineering Inc, Canada). RH microsuspension was prepared using 1:0.5:100 drug/poloxamer 188/water ratio (w/w).

2.5. Preparation of RH nanosuspension

RH nanosuspension was produced by high pressure homogenization using an AH 100 apparatus (ATS Engineering Inc, Canada) as well. Before producing RH nanosuspension, the RH coarse powder was grinded in a mortar for 10 min and then dispersed in 0.5% (w/w) poloxamer 188 bidistilled water solutions. The obtained suspension was placed in an ultrasonic water bath (Shenyang Ultrasonic Technology Co. Ltd., Shenyang, China) for sonication for 20 min and finally the gained coarse suspension was homogenized at high pressure by high pressure homogenization. The nanosuspension was produced applying 2 cycles at 90 bar, 2 cycles at 200 bar, 2 cycles at 500 bar and 15 cycles at 800 bar. The drug/poloxamer 188/water ratio was 1:0.5:100 (w/w).

2.6. Lyophilization

RH microsuspension and nanosuspension were dried using lyophilization for further physicochemical characterization.

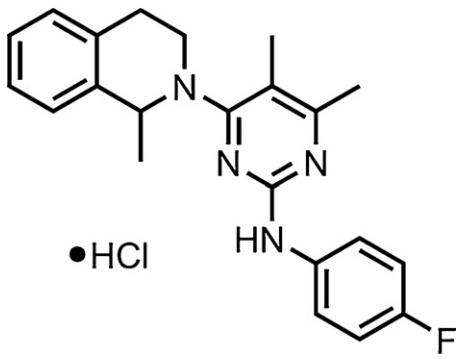

Fig. 1. Chemical structure of revaprazan hydrochloride.

Approximately 1 ml of the aqueous suspensions were frozen at −80 °C in a 10 ml vial for 24 h. Then the frozen suspensions were freeze dried using an EYELA FDU-1100 freeze drier (Tokyo Rikakikai Co., Ltd., Japan) for 24 h, without applying a secondary drying process.

2.7. Particle size analyses

The average diameter (*Z*-AVE) and polydispersity index (PI) of RH coarse suspension, microsuspension and nanosuspension were determined by photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern Instruments, UK) at room temperature. Laser diffraction (LD) (Coulter LS 230, Beckman-Coulter, USA) was employed additionally to measure particle size distribution of the three RH suspensions. Each sample was measured three times.

2.8. Scanning electron microscopy (SEM)

The morphology of coarse RH powder and the freeze dried samples were determined using scanning electron microscopy (SEM) (S-4800, Hitachi Technologies Corporation, Japan). Prior to examination, the samples were mounted onto metal stubs using a double sided adhesive tape and sputtered with a thin layer of gold under vacuum. The scanning electron microscope was operated at an acceleration voltage of 1.5 kV.

2.9. Differential scanning calorimetry (DSC)

DSC measurements were performed using a Mettler-Toledo thermal analyzer (DSC 1, Mettler-Toledo International Inc., Switzerland). Samples (RH coarse powder, poloxamer 188 powder, RH/poloxamer 188 physical mixture, the freeze dried microsuspension powder and nanosuspension powder) were placed in a standard aluminium crucible fitted with a perforated lid for scanning. A heating rate of 10 °C/min was employed in the range of 25–260 °C and an empty pan was adopted as reference.

2.10. Powder X-ray diffraction (PXRD)

Powder X-ray diffractometer (D/Max-2500PC, Rigaku, Japan) was used for X-ray diffraction studies. The scanning rate was 4°/min in the range $3^\circ \leq 2\theta \leq 55^\circ$. Samples investigated using PXRD analysis were the same batch as those used in DSC analysis.

2.11. In vitro dissolution test

The dissolution tests of the three suspensions were carried out in 900 ml phosphate buffer solution (pH 7.4) containing 0.02% (v/v) Tween 80 by USP Apparatus 2 employing a dissolution tester (ZRS-12G, Tianjin TDTF Technology Co., Ltd, China). 2.5 ml suspension

(1%, w/v) samples were introduced directly into the vessels. Rotating speed and bath temperature were maintained at 100 rpm and 37.0 ± 0.5 °C, respectively. 5 ml samples were withdrawn at certain time intervals, and filtered through 0.45 μm filter. The amount of RH dissolved was determined by HPLC.

2.12. In vivo studies

2.12.1. In vivo studies in rats

Male Wistar rats (weighted 180–220 g, 8–10 weeks old) were used as experiment animals. Eighteen rats were divided into three groups (six each). Before administration the rats were fasted over night with free access to water. The next morning, RH suspensions (coarse suspension, microsuspension, nanosuspension), 50 mg/kg, were administered orally to rats in the three groups, respectively. Blood samples (0.1–0.2 ml) were collected via the jugular vein at 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720, and 1440 min after administration. The collected blood samples were placed in dried heparinized Eppendorf tube and then separated immediately by centrifugation at 4000 rpm for 10 min and stored at −20 °C until analysis (Han et al., 1998). All experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and also in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University.

2.12.2. Plasma sample preparation

Deproteinization method was employed. 10 μl internal standard solution (Vinpocetine methanol solution, 4 μg/ml) and 115 μl acetonitrile were added to each 50 μl rat blood sample. The mixtures were vortexed (MS3, IKA®, Germany) for 3 min and centrifugated (H 2050R, Xiang Yi Centrifuge Instrument Co., Ltd, Hunan, China) at 9000 × *g* for 10 min. Thereafter 20 μl volume of the supernatant was directly injected onto the HPLC column for analysis (Han et al., 1997).

2.13. Analytical method

2.13.1. Quantitative analysis of RH from dissolution media

The concentrations of RH were measured by HPLC. The HPLC equipment consisted of a Hitachi 7100 pump, a Hitachi UV–vis L-7420 detector (both from Hitachi Technologies Corporation, Japan) set at 270 nm and an HT-220A column temperature controller (Model320, Tianjin Pu Xiang Science & Technology Co., Ltd., China). Analyses were performed on a 5 μm ODS column (200 mm × 4.6 mm, DIKMA Technologies, China) operated at 45 °C. The mobile phase, methanol–water system (93:7), ran at 1.0 ml/min and the retention time of the drug was approximately 6 min.

2.13.2. Quantitative analysis from plasma

RH in plasma was determined by the same HPLC system and the same ODS column described above using vinpocetine as internal standard. A mobile phase containing 0.015 M (w/v) ammonium dihydrogen phosphate in methanol–water mixture (89:11, v/v) was delivered at 1.0 ml/min. The retention times of the internal standard and drug were approximately 7 min and 9 min, respectively.

2.14. Pharmacokinetic data analysis

Pharmacokinetic data analysis was carried out using drug and statistics software (DAS® 2.0, Boying Corporation, China). Results were expressed as a mean ± SD. Analysis of variance (ANOVA) test was performed to demonstrate statistical differences using SPSS 16.0 software (SPSS, Chicago, IL, USA). *P* value less than 0.05 was considered significant.

Table 1
PCS (photon correlation spectroscopy) particle size data of RH coarse suspension, microsuspension and nanosuspension (PI, polydispersity index) ($n = 3$).

	Z-AVE (nm)	PI
Coarse suspension	2154.50 ± 209.10	1.00
Microsuspension	1481.60 ± 93.30	1.00
Nanosuspension	562.30 ± 16.00	0.30 ± 0.05

3. Results and discussion

3.1. Particle size analysis and scanning electron microscopy (SEM)

Particle size distribution of the three RH suspensions was measured by photon correlation spectroscopy (PCS) and laser diffraction (LD). The PCS diameter (*Z*-AVE) and polydispersity index (PI) of the three suspensions are reported in Table 1. And the results of LD measurements are shown in Table 2. These data were corresponding to the information gained from SEM images in Fig. 2.

Photon correlation spectroscopy (PCS) yields the intensity weighted average diameter of the bulk population (*Z*-AVE) and the polydispersity index (PI) as measures for the width of distribution. The results of laser diffraction (LD) were calculated as volume distribution with the optical parameters 1.593 for real refractive index and 0.01 for the imaginary refractive index. Data in Tables 1 and 2 showed RH nanosuspension gained narrow size distribution, whereas RH microsuspension and coarse suspension were widely distributed. The PI values of RH microsuspension and coarse suspension were recorded at 1.00 which indicated the particle size distribution was too wide and the data may not display the true situation. Because the larger particles in the two suspensions were already precipitated, these data only showed the particle size distribution of the ones suspended in the solution.

These particle size data and SEM images indicated that particles in the three suspensions were different not only in size, but also in shape, which would influence their dissolution behavior and bioavailability (Mosharraf and Nyström, 1995). The freeze dried RH nanosuspensions were needle like, while the freeze dried microsuspensions were longer and wider, and the coarse powders had a granular shape.

3.2. Thermal analyses by DSC

To study crystalline state of different sized drug particles, thermal analysis of RH coarse powder, poloxamer 188 powder, RH/poloxamer 188 physical mixtures, freeze dried RH microsuspension and nanosuspension powder were performed.

Information of thermal analysis by DSC can be seen in Fig. 3 and Table 3. In DSC measurements, crude drug powder showed the melting endotherm at 222.04 °C, while the melting endotherm of

Table 2
LD (laser diffraction) particle size data of RH coarse suspension, microsuspension and nanosuspension ($n = 3$).

	Mean size (μm)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
Coarse suspension	47.47	3.68	44.39	95.50
Microsuspension	2.77	0.53	2.09	5.89
Nanosuspension	0.64	0.43	0.62	0.88

Table 3
Melting point and enthalpy value of different sized RH powders.

Parameter	Nanocrystal	Microcrystal	Physical mixture	Coarse powder
Melting point (°C)	191.77	201.04	220.26	222.04
Melting enthalpy (kJ/mol)	12.08	21.76	31.27	41.69

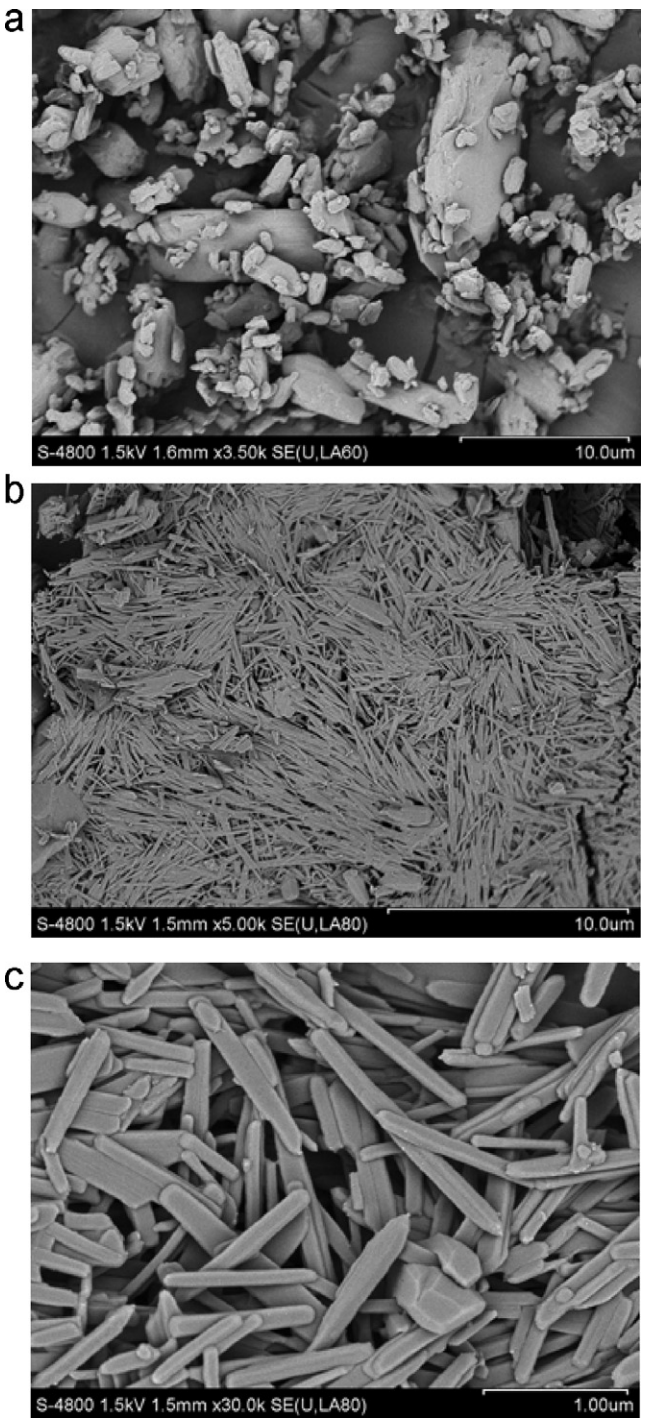

Fig. 2. SEM images of RH coarse powder (A), freeze dried microsuspension powder (B) and freeze dried nanosuspension powder (C).

freeze dried drug nanosuspension powder, microsuspension powder and the physical mixture were recorded at 191.77 °C, 201.04 °C, and 220.26 °C, respectively. Poloxamer 188 powder showed the melting point at 54.91 °C, however, the melting point of poloxamer 188 in freeze dried drug nanosuspension powder, microsuspension powder and the physical mixture were recorded at 48.26 °C, 52.42 °C, and 55.05 °C, respectively. Since the melting point of drug and poloxamer 188 in the physical mixture did not show any significant differences with that of crude RH powder and poloxamer 188, the decrease of the melting endotherm of RH nanoparticles and microparticles was due to the effect of particle size reduction rather than the attendance of poloxamer 188 in the formulation.

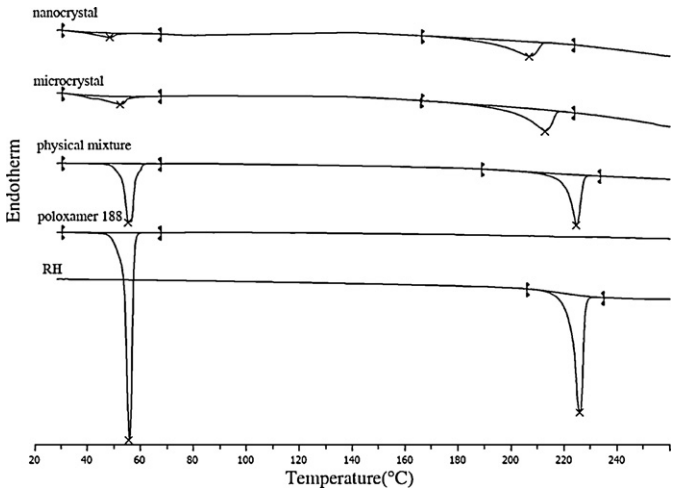

Fig. 3. DSC profiles: from top to bottom were, in order, freeze dried nanosuspension powder, freeze dried microsuspension powder, RH powder/poloxamer 188 physical mixture, poloxamer188 powder and coarse RH powder.

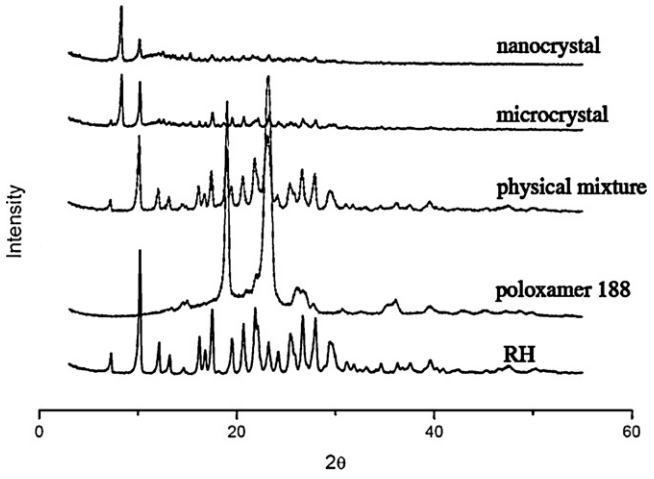

Fig. 4. PXRD patterns: from top to bottom were, in order, freeze dried nanosuspension powder, freeze dried microsuspension powder, RH powder/poloxamer 188 physical mixture, poloxamer 188 powder and coarse RH powder.

Analysis of melting point indicated no change between coarse powder and physical mixture. Yet analysis of melting enthalpy indicated a decrease for physical mixture. This was probably caused by the preparation processes. During the preparation procedure (RH powder and poloxamer 188 were blended until a homogenous mixture was obtained) some certain amount of smaller particles could be produced. Therefore, the physical mixture may display certain properties of nanoparticle. The integral normalized enthalpy value decreased with decrease of RH particle size. Low melting point and enthalpy value mean low lattice energy and particles with lower lattice energy are easier to dissolve.

3.3. Powder X-ray diffraction (PXRD) analyses

To confirm crystalline state of freeze dried RH nanosuspension and microsuspension, powder X-ray diffraction was performed with the same batch of samples that were measured by DSC. All PXRD patterns are displayed in Fig. 4. From the diffractograms we could see that there were no differences in peak position of the drug among these samples. Accordingly all freeze dried nanosuspension powders and microsuspension powders were in the same crystalline state as the raw material. All kinds of energy input during production did not change their crystalline state, either the attendance of stabilizer. However, distinctions in relative peak intensity

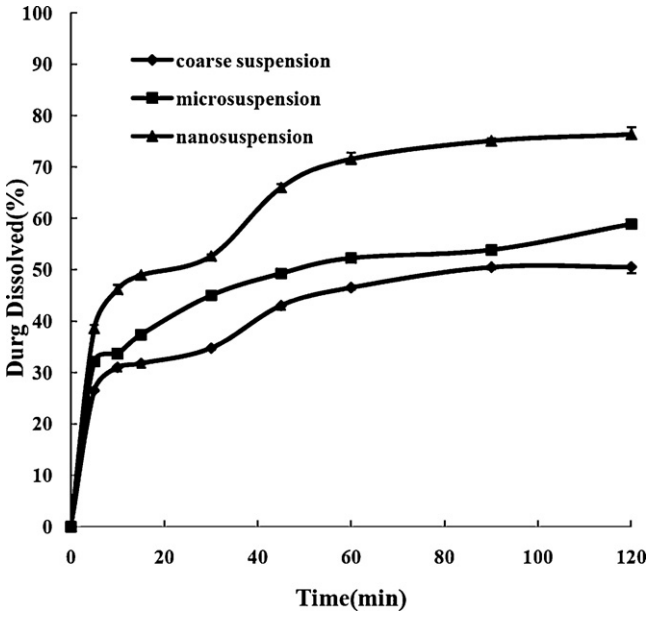

Fig. 5. Dissolution profiles of RH coarse suspension, microsuspension and nanosuspension.

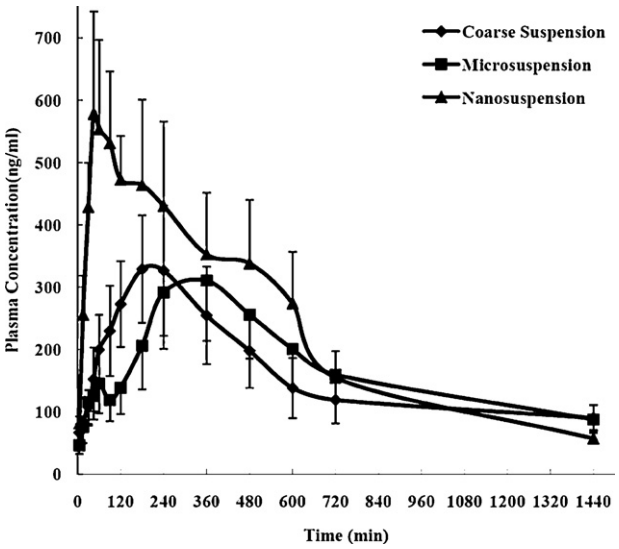

Fig. 6. Plasma concentration–time profiles of coarse suspension, microsuspension and nanosuspension.

can be detected among the samples. That was probably caused by particle size reduction. Usually, drugs with lower crystallinity and smaller size have higher dissolution rate and bioavailability (Sarkari et al., 2002; Zhong et al., 2005). Therefore, particle size reduction and the change in crystallinity of RH powder were expected to increase its dissolution rate and bioavailability.

3.4. In vitro dissolution test

The dissolution profiles of RH coarse suspension, microsuspension and nanosuspension are shown in Fig. 5. The dissolution rates of the three suspensions were approximately 50.53%, 58.90% and 76.34% in 120 min, respectively, which suggested that dissolution of RH from the three suspensions followed Noyes–Whitney equation (Noyes and Whitney, 1897); particle size reduction could lead to enhancement in dissolution rate. Though the microsuspension and nanosuspension showed increased dissolution rate, the extent increased was not that significant compared with other researchers' work (Hecq et al., 2005, 2006; Kim et al., 2008; de Waard et al., 2008; Mauludin et al., 2009). This can be explained by the influence of both the particle size and shape on the dissolution rate. The Noyes–Whitney equation:

$$\frac{dC}{dt} = D \cdot S \cdot \frac{C_s - C_t}{h} \quad (1)$$

where dC/dt represents the dissolution rate, D is the diffusion coefficient of the solute, h denotes the thickness of the dissolution boundary layer and S represents the surface area, C_s is the saturation solubility and C_t the bulk concentration. It is widely accepted that the enhancement in dissolution of drug can be attributed to the particle size reduction, especially to the nano range which provide a strong increase in surface area available to dissolve. Besides the size factor, the particle shape also plays an important role in affecting drug dissolution. Long, flaky particles with a high degree of irregularity may cause an increase in the average hydrodynamic boundary layer thickness. In that case the h value would increase and as a result the dissolution rate would decrease (Mosharraf and Nyström, 1995).

It can be seen from the SEM images of different sized RH particles in Fig. 2, none of them are spherical. Accordingly, the dissolution enhancement was probably only owing to their size reduction. For RH microsuspension and nanosuspension, although they had needle like particles, their particle size were smaller than the coarse suspension, so they had much more surface area. That might be the reason why they owned higher dissolution rate.

3.5. Bioavailability study in rats

The plasma concentration–time curves of RH suspensions are shown in Fig. 6, and the pharmacokinetic parameters are displayed in Table 4. As shown in these data three suspensions were different from each other in the corresponding parameters. In contrast with the RH coarse suspension and microsuspension, the nanosuspension had higher mean C_{max} (87.23% and 97.70% higher), AUC_{0-t} (45.23% and 36.03% higher) and shorter T_{max} (185 min and 315 min shorter), MRT (114 min and 157 min shorter), which indicated RH nanosuspension was easier to be absorbed. When it came to coarse suspension and microsuspension, there were no significant differences between the two groups concerning these parameters mentioned above.

The reasons for these phenomena were probably caused by the absorption pattern of RH. The uptake of RH into the Caco-2 cell monolayer appeared to be mediated by a high-affinity transporter (Li et al., 2001). More exposure of drug function group would help uptake of drug. Therefore, particle size has been recognized as a crucial parameter for bioadhesion to and absorption from gastrointestinal tissue (Jung et al., 2000). The dissolution profiles

Table 4
Pharmacokinetic parameters of RH coarse suspension, microsuspension and nanosuspension after oral administration in rats ($n = 6$).

Parameter	Coarse suspension	Microsuspension	Nanosuspension
C_{max} (μg/ml)	0.37 ± 0.09	0.35 ± 0.11[a]	0.69 ± 0.09[b]
AUC_{0-t} (min μg/ml)	231.74 ± 46.39	247.39 ± 71.46[a]	336.55 ± 107.00[b]
T_{max} (min)	230 ± 70.14	360 ± 107.33[a]	45 ± 16.52[b]
MRT (min)	551.03 ± 62.76	594.60 ± 57.47[a]	437.11 ± 61.69[b]

Results were expressed as the mean ± SD. a: $P > 0.05$; b: $P < 0.05$, compared to the corresponding parameters of RH coarse suspension.

showed that drug nanosuspension had the highest dissolution rate among the three groups. Since drug nanoparticles had much more surfaces under the same condition, they could dissolve easily into intestinal fluid and smaller particles show a higher extent of uptake than larger ones via both follicle associated epithelia and absorptive enterocytes (Jani et al., 1989, 1990). RH nanosuspension was absorbed easily which led to increased AUC_{0-t}, C_{max} and decreased T_{max}, MRT.

For microsuspension group, its AUC_{0-t} and C_{max} were expected to be larger than coarse suspension group according to the *in vitro* dissolution test. Nevertheless, in fact, there were no significant differences between the two groups concerning the parameters mentioned above and the crude powder suspension group even had higher plasma concentration at certain initial time points. That may be caused by the following reasons: (i) as we could see from the LD particle size data and SEM images of crude powder, its particle size distribution was rather wide; a few drug particles were even in nano range. And because of the poor water solubility of RH, the nano sized particles could make great contributions to enlarge AUC_{0-t} and C_{max}. And that is also why T_{max} and MRT of coarse suspension were almost the same with that of microsuspension. (ii) Particle surface properties, such as surface charge and hydrophile–lipophile balance, have important impacts on gastrointestinal absorption of drug. Negative charged particles combined with hydrophilic matrix materials highly increased bioadhesive properties (Hillery and Florence, 1996; Jung et al., 2000) and further increased drug absorption in GI tract. The stabilizer, poloxamer 188, is one of the hydrophilic excipients, and in GI tract, RH is positive charged. As a result the combination of the hydrophilic poloxamer 188 and the positive charged RH may not lead to well drug absorption in GI tract. The microsuspension group did not show its superiority of smaller particle size markedly. On the other hand because of existence of the few nano sized particles, absorption of RH coarse suspensions in GI tract was not that low. In the case of nanosuspension, though stabilized by poloxamer 188, fast absorption was evident and obvious, and the only difference among the three RH suspensions was their particle size. All these once more demonstrated the importance of size reduction on enhancing drug absorption.

4. Conclusions

RH nanosuspension and microsuspension were prepared by high pressure homogenization. Their crystalline state were evaluated by DSC and PXRD, and both evaluations indicated lattice energy of drug particles decreased with decrease of particle size. In the present study, it had been shown that particle size reduction could increase RH *in vitro* dissolution rate. The smaller the particle size, the higher the dissolution rate. While unlike the *in vitro* evaluations, *in vivo* studies indicated that only nanosuspension could significantly increase oral bioavailability of RH in rats. That means, in the case of particle size reduction, enhanced oral bioavailability can be achieved by reducing RH particle size into nano range.

Acknowledgments

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References

de Waard, H., Hinrichs, W.L.J., Frijlink, H.W., 2008. A novel bottom-up process to produce drug nanocrystals: controlled crystallization during freeze-drying. J. Control. Release 128, 179–183.

Han, K.S., Choi, H.C., Yoo, J.K., Lee, J.W., Lee, M.G., 1997. Determination of a new proton pump inhibitor YH1885, in plasma and urine by high-performance liquid chromatography. J. Chromatogr. B 696, 312–316.

Han, K.S., Kim, Y.G., Yoo, J.K., Lee, J.W., Lee, M.G., 1998. Pharmacokinetics of a new reversible proton pump inhibitor. YH1885, after intravenous and oral administrations to rats and dogs: hepatic first-pass effect in rats. Biopharm. Drug Dispos. 19, 493–500.

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., Amighi, K., 2005. Preparation and 398 characterization of nanocrystals for solubility and dissolution rate enhancement of 399 nifedipine. Int. J. Pharm. 299, 167–177.

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., Boulanger, P., Le, L.S., Amighi, K., 2006. Preparation and in vitro/in vivo evaluation of nano-sized crystals for dissolution rate enhancement of ucb-35440-3, a highly dosed poorly water-soluble weak base. Eur. J. Pharm. Biopharm. 64, 360–368.

Hillery, A.M., Florence, A.T., 1996. The effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60-nm polystyrene particles after oral administration in the rat. Int. J. Pharm. 132, 123–130.

Hwang, M.S., Lee, S.J., Song, K.S., Kim, C.S., Lee, B.Y., Lee, J.W., 1998. Kinetic studies of a novel reversible pump antagonist, YH 1885, on H^+/K^+-ATPase. In: The Spring Convention of the Pharmaceutical Society of Korea , Pusan, April 24–25, 1998.

Jani, P., Halbert, G.W., Langridge, J., Florence, A.T., 1989. The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. J. Pharm. Pharmacol. 41, 809–812.

Jani, P., Halbert, G.W., Langridge, J., Florence, A.T., 1990. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol. 42, 821–826.

Jinno, J., Kamada, N., Miyake, M., Yamada, K., Mukai, T., Odomi, M., Toguchi, H., Liversidge, G.G., Higaki, K., Kimura, T., 2006. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J. Control. Release 111, 56–64.

Jung, T., Kamm, W., Breitenbach, A., Kaiserling, E., Xiao, J.X., Kissel, T., 2000. Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? Eur. J. Pharm. Biopharm. 50, 147–160.

Kim, M.S., Jin, S.J., Kim, J.S., Park, H.J., Song, H.S., Neubert, R.H., Hwang, S.J., 2008. Preparation, characterization and in vivo evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. Eur. J. Pharm. Biopharm. 69, 454–465.

Li, H., Chung, S.J., Kim, D.C., Kim, H.S., Lee, J.W., Shim, C.K., 2001. The transport of a reversible proton pump antagonist 5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl) pyrimidine hydrochloride (yh1885), across caco-2 cell monolayers. Drug Metab. Dispos. 29, 54–59.

Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int. J. Pharm. 125, 309–313.

Loftsson, T., Brewster, M.E., Mässon, M., 2004. Role of cyclodextrins in improving oral drug delivery. Am. J. Drug. Deliv. 2, 261–275.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Makhlof, A., Miyazaki, Y., Tozuka, Y., Takeuchi, H., 2008. Cyclodextrins as stabilizers for the preparation of drug nanocrystals by the emulsion solvent diffusion method. Int. J. Pharm. 357, 280–285.

Mauludin, R., Müller, R.H., Keck, C.M., 2009. Kinetic solubility and dissolution velocity of rutin nanocrystals. Eur. J. Pharm. Sci. 36, 502–519.

Mosharraf, M., Nyström, C., 1995. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. Int. J. Pharm. 122, 35–47.

Müller, R.H., Akkar, A., 2004. Drug nanocrystals of poorly soluble drugs. Encyclop. Nanosci. Nanotechnol. 2, 627–638.

Müller, R.H., Bohn, B.H.L., 1998. Nanosuspensions. In: Müller, R.H., Bohn, B.H.L., Benita, S. (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs. Medpharm, Stuttgart.

Noyes, A.A., Whitney, W.R., 1897. The rate of solution of solid substances in their own solutions. J. Am. Chem. Soc. 19, 930–934.

Park, Y.J., Hyun, C.K., 2008. Revaprazan-containing solid dispersion and process for the preparation thereof. WO Patent 2008/078922.

Sarkari, M., Brown, J., Chen, X., Swinnea, S., Williams, R.O., Johnston, K.P., 2002. Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int. J. Pharm. 243, 17–31, 3rd.

Stovall, D.M., Givens, C., Keown, S., Hoover, K.R., Barnes, R., Harris, C., Lozano, J., Nguyen, M., Rodriguez, E., Acree, J.W.E., Abraham, M.H., 2005. Solubility of crystalline nonelectrolyte solutes in organic solvents: mathematical correlation of 4-chloro-3-nitrobenzoic acid and 2-chloro-5-nitrobenzoic acid solubilities with the Abraham solvation parameter model. Phys. Chem. Liq. 43, 351–360.

Tao, T., Zhao, Y., Wu, J., Zhou, B., 2009. Preparation and evaluation of itraconazole dihydrochloride for the solubility and dissolution rate enhancement. Int. J. Pharm. 367, 109–114.

Wang, Z., Deng, Y., Sun, S., Zhang, X., 2006. Preparation of hydrophobic drugs cyclodextrin complex by lyophilization monophase solution. Drug. Dev. Ind. Pharm. 32, 73–83.

Zhong, J., Shen, Z., Yang, Y., Chen, J., 2005. Preparation and characterization of uniform nanosized cephradine by combination of reactive precipitation and liquid anti-solvent precipitation under high gravity environment. Int. J. Pharm. 301, 283–293.