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Characterization and Bioavailability of Wogonin by Different Administration Routes in Beagles

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACE 1 **Na Zhu***
BCF 1 **Jian-chun Li***
ACD 2 **Jin-xiu Zhu**
CFG 1 **Xiu Wang**
BDG 1 **Jin Zhang**

1 Faculty of Pharmacy, Bengbu Medical College, Bengbu, Anhui, P.R. China

2 Department of Pharmacy, The 1st Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui, P.R. China

Corresponding Author:

* These authors contributed equally to this work

Jian-Chun Li, e-mail: liewelloo@163.com

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Background:

With the gradually accumulating research on pharmacological activity of wogonin, the *in vitro* analysis research on wogonin has become more and more popular, but there are very few reports about *in vivo* detection, and there are no solid dispersions (SDs) of Wogonin. The aim of this study was to explore the formation of solid dispersions (SDs) of wogonin. The reasons for the low bioavailability were studied through different routes of administration.

Material/Methods:

SDs was formulated using the solvent evaporation method via polyvinylpyrrolidone K30 (PVP). The characterization of the drug and its carrier was detected by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The serum concentrations of Wogonin were detected using the LC-MS/MS method. Six beagles were fed 3 different formulations of wogonin in 3 cycles.

Results:

The SDs of wogonin had a higher solubility than the physical mixtures. Based on XRD and DSC, wogonin was transformed from a crystalline morphology to an amorphous structure. The main pharmacokinetic parameters of i.g. administration (crude material and SD) and i.v. route were as follows: C_{max} (2.5 ± 1.1), (7.9 ± 3.3), and (6838.7 ± 1322.1) $\mu\text{g/L}$, t_{max} (0.7 ± 0.3) and (0.3 ± 0.2) h for the former, AUC_{0-t} (7.1 ± 2.0), (21.0 ± 3.2) and (629.7 ± 111.8) $\mu\text{g}\cdot\text{h/L}$. The absolute bioavailability of native wogonin and wogonin arginine solution were (0.59 ± 0.35)% and (3.65 ± 2.60)%. Further research showed that the low bioavailability of wogonin might be associated with low solubility and rapid combination with glucuronic acid *in vivo*.

Conclusions:

The significantly increased solubility of SDs and the further preparation of arginine solution could significantly increase the bioavailability of wogonin.

MeSH Keywords:

Biological Availability • Dogs • Solid-Phase Synthesis Techniques • Tandem Mass Spectrometry

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Background

Oral administration is still the optimal route to administer medication, especially for chronic diseases which need long-term treatment, and intravenous administration is not readily accepted by patients. Oral administration is the simplest and most effective route of treatment [1,2]; however, some oral drugs are poorly soluble, so oral bioavailability is very low, which limits its application. Therefore, increasing the dose and changing the internal structure has become essential in improving the low bioavailability [3–5].

Wogonin has a variety of pharmacologic effects and biological activities, including anti-cancer [6], anti-viral [7], anti-oxidative [8], anti-inflammatory [9], and anti-seizure [10] effects. Moreover, wogonin is used as remedial treatment in traditional Chinese medicine for fever, cough, respiratory tract inflammation, and hypotension, which could relieve patient discomfort, provide individualized treatment, and ensure compliance. Previous studies have shown that wogonin has potential in the prevention and treatment of tumors [11–13].

After oral administration, absorption in the gastrointestinal tract and hepatic metabolism and intake are needed to ensure entry of the drug in the body; however, the water solubility of wogonin is very low, which limits its application. The intrinsic physicochemical properties and low chemical stability of wogonin limits its bioavailability and further research and development. Therefore, it is very important to improve water solubility [4,14,15].

Improvement in solubility could promote drug absorption and curative effect. To improve the solubility and bioavailability of insoluble drugs, researchers have found a variety of processing technologies, such as micronization, solubilization, salt formation, use of surfactants, complex formation with polymers, and alterations in pH [3,4,16–20]. Among various approaches, solid dispersion (SD) technology is promising in the application of low solubility drugs because SD technology has a number of advantages, is easy to use, and is economical. Increased wettability and reduced homogeneous distribution of a small amount of drug in the solid state and increased the interfacial area [21–23]. Various hydrophilic carriers are often used in SD preparation, such as polyethylene glycol, polyvinylpyrrolidone (PVP), labrasol, and poloxamers, which are used to improve the dissolution rate and bioavailability of drugs with poor water solubility. In the preparation of SD, the solvent method can be applied in mass production [14,16,21,24–26].

To the best of our knowledge, there are no reports on the detection of wogonin in beagles using the LC-MS/MS method. According to the literature [27], by using HPLC detection, the minimum quantitative lower limit reached only 20 ng/mL, and

was detected only in blood collected 5 h after the treatment, which does not meet the biological sample detection method 3–5 half-life. Therefore, in the current study the LC-MS/MS method was successfully used in the determination of wogonin in the blood of beagles [28]. SD has been widely and successfully used in improving the solubility and bioavailability of insoluble drugs [16,26,29,30]. To further explore the reasons for the low long-term level of the parent drug in the body, experiments involving the administration of a wogonin arginine solution were carried out. The reason for the low bioavailability of Wogonin was investigated to provide a reference for the continued study on the dosage form and clinical use.

Material and Methods

Materials

Wogonin (purity >99%) and the solution preparation (10 g/L, wogonin arginine) were purchased from Heyuan Con-source Medicine Technology Co., Ltd., China. PVP-K30 was ordered from Zhongmei Huadong Pharmaceutical Technology Co. Ltd. (Hangzhou, China) and β -glucuronidase was ordered from (Sigma, USA). HPLC-grade methanol was obtained from Merck (Darmstadt, Germany). Formic acid (DIMA, USA) was of HPLC-grade and acetic ether was commercially available and of analytical grade. Distilled water processed using the Millipore purifying system was used throughout this study.

Animals

Six male beagle dogs, with the average weight of 8.2 ± 0.4 kg were obtained from the Jia'an Animal Experimentation Breeding Center (Zhejiang, China). The animals were cared for according to the regulations of the Animal Committee at the constant temperature of $22 \pm 1^\circ\text{C}$, 12-h/12-h light-dark cycle and 10–15 air changes per h for the feeding room. The studies were approved by the Animal Ethics Committee of Anhui Medical University.

Analytical system

The data analysis was done using the Finnigan TSQ Quantum Discovery MAX system with ESI interface and Xcalibur workstation software (Ver. 1.4) for data processing. Isocratic elution used a mobile phase of methanol-water containing 0.1% formic acid (70: 30) and the mobile phase was ultrasonicated first before being filtered through a $0.45\text{-}\mu\text{m}$ Millipore filter. Separation was carried out on a Phenomenex® Luna C18(2) column (150×2.0 mm, $5\text{ }\mu\text{m}$, Code No. 490902-2) with a C18 guard column (Phenomenex, USA). The treated supernatant was transferred to an autosampler vial, and $10\text{ }\mu\text{L}$ was injected into the column for analysis. The flow rate was 0.2 mL/min

and all the analyses were performed at 40°C. The optimized MS parameters were selected as follows: Spray Voltage 4.5 kV; Capillary Temperature 320°C; Source CID -4V; Sheath Gas Pressure 35 Arb; Aux Gas Pressure 5 Arb; Collision Pressure 1.2. Multiple-reaction monitoring (MRM) used the transitions of the protonated molecules at m/z 285.0→270.0 for wogonin and 373.3→305.3 for finasteride with scan time of 0.5 s per transition.

Preparation of the physical mixture and SD

A specific amount of crude material and some of the carriers were completely crushed and mixed to form the physical mixture, and stored in a dry container for further analysis. SD was prepared using the solvent method. The crude wogonin material and carrier were prepared, weighed, and dissolved in a sufficient amount of anhydrous alcohol. According to the dilution method, the mixture was added to the excipients.

Characterization of SD

XRD analysis and detection

PVP K30, wogonin, the physical mixture, and SD were analyzed with X-ray diffraction analysis. The running conditions were as follows: Cu target; graphite monochromator monochromatized diffraction; tube voltage, 40 kV; and tube current, 40 mA.

DSC analysis and detection

PVP K30, wogonin, the physical mixture, and SD were analyzed with DSC analysis. The running conditions were as follows: the empty crucible was used as a reference aluminum pool; another empty crucible was used as a sample pool; approximately 10-mg samples were added; the scanning speed was 5°C/min; and the scanning range was 53–303°C.

Dissolution analysis

In vitro dissolution studies were performed using a USP dissolution device. A specific amount of wogonin crude drug and accessories (PVP K30) was dissolved in anhydrous ethanol, mixed, ultrasonic-treated, and depression vaporized in a 60°C water bath. After the solvent was completely dry, it was put in a dry container, and vacuum dried for 24 h. The solid was gently scraped, smashed, filtered through 80 mesh, and stored in a dry container.

Study on pharmacokinetics and bioavailability

Six male beagles were introduced and randomly divided into 3 groups. Three periodic cross tests were performed in 3 preparations. All dogs were fasting, but water was permitted 12

h before the experiments. In the first cycle, drug was injected in group I via the small saphenous vein of the hind leg or ear vein (5 mL/kg, injection within 1 min, and at a constant speed). Three milliliters of blood of the contralateral hind limb vein was obtained before the administration of drug and at 0, 5, 10, 20, 30, 45, and 60 min, and 1.5, 2, 3, 4, 5, 6, 8, and 12 h after administration. Drug was administered intragastrically (i.g.) to group II (wogonin crude drug [15 mg/kg], mixed with 0.5% sodium carboxymethyl cellulose sodium solution). Drug was administered intragastrically to group III (solid dispersion of wogonin [5 mg/kg], mixed with 0.5% sodium carboxymethyl cellulose sodium solution). We used 20 mL of cold water to rinse the stomach tube after intragastric administration. Blood samples from the hind limb vein were obtained before the administration and at 0, 5, 10, 20, 30, 45, and 60 min, and 1.5, 2, 3, 4, 6, 8, 12, 24, and 36 h after administration, placed in the centrifuge tube, centrifuged at 4000 rpm for 10 min to isolate plasma, and stored at –20°C until analysis. After a washout period of 1 week, the next cycle of administration was carried out. The blood sampling time points were the same as with the first cycle.

Results

Characterization of SD

XRD analysis

PVP K30, wogonin, the physical mixture, and SD were analyzed by X-ray diffraction. Figure 1A shows crude materials of wogonin has a characteristic crystallization peak under XRD with many crystal diffraction peaks evident. PVP K30 is a kind of amorphous material without characteristic peaks within the range of 5–50°C (Figure 1B). A physical mixture with PVP K30 can decrease the characteristic crystallization peak of wogonin to a certain extent (Figure 1C). The form of the drug and physical mixture have obvious differences. After the SD was made, the crystallization peak of wogonin completely disappeared (Figure 1D). We can conclude the wogonin exists in molecular states in supplementary material, which indicates that wogonin existed in an amorphous state in SD.

DSC analysis

DSC is considered to be a modern and accurate technique used in solid dispersion analysis. Figure 2A shows that the crystallization peak of wogonin has a sensitive crystal endothermic peak at 193.58°C. In the wogonin-PVP K30 (1:5) physical mixture, the characteristic absorption peak of wogonin at 193.58°C disappeared (Figure 2C). There is a weak endothermic peak at 148.16°C, which is the result of wogonin endothermic peak forward due to the crystalline form change of wogonin caused

Figure 1. X- ray diffraction (A) wogonin; (B) PVP K30 wogonin crude material; (C) Wogonin PVP K30 physical mixture (1:5); (D) Wogonin-PVP K30 solid dispersion (1:5).

by hydrogen bond formation between drug and carrier during the heating process. The characteristic melting peak of wogonin in wogonin-PVP K30 (1:5) SD complete obliteration is not totally absent. There was no obvious endothermic or exothermic peak in the entire process (Figure 2B), which means that there was no drug crystal co-precipitate and wogonin exists in an amorphous state dispersed in the carrier.

Solubility study *in vitro*

The study on solubility *in vitro* showed that the dissolution rate of the SD of wogonin was significantly increased compared with the physical mixture and the crude material. The study also showed that when the drug loading ratio of SD was 1:5, the percentage of drug dissolution was 10.2 times the physical mixture at 10 min. The percentage of drug accumulation dissolution rate was >80%, which was 7 times more than with the physical mixture. It is generally well known that a drug in a solid dispersions system sometimes exists in an amorphous form. The amorphous form of a drug has a higher thermodynamic activity than its crystalline form, leading to rapid dissolution of the drug. The improved drug release rate could be

attributed to the drug crystallinity reduction in the wogonin-loaded solid dispersions prepared by PVP K30. Dissolution rate improvement for PMs may be mostly related to the hydrophilic effect of PVP. This hydrophilic polymer can reduce the interfacial tension between wogonin and the release medium and provide greater dissolution rates.

Study on pharmacokinetics and bioavailability

Study on the bioavailability of 3 types of drug delivery methods

The 3 routes of medication administration were intravenous (arginine salt solution), gastric (crude drug), and gastric (SD). The main pharmacokinetic parameters were as follows: C_{max} (2.5 ± 1.1), (7.9 ± 3.3), and (6838.7 ± 1322.1) $\mu\text{g/L}$; t_{max} (0.7 ± 0.3) and (0.3 ± 0.2) h for the former; and AUC_{0-t} (7.1 ± 2.0), (21.0 ± 3.2), and (629.7 ± 111.8) $\mu\text{g}\cdot\text{h/L}$. The difference in AUC was very large between the different drug delivery methods; the individual difference in $t_{1/2z}$ was large, but the average value of different methods was not statistically significant ($P > 0.05$), which indicates that the elimination of wogonin was similar in the

Figure 2. Differential X-ray diffraction (A) PVP K30; (B) Wogonin crude material; (C) Physical mixture; (D) Solid dispersion, wogonin-PVP K30 (1:5).

different routes of administration. Intragastric administration of SD was absorbed faster in dogs than the crude drug. The C_{max} increased nearly 10 times compared with crude drug after dose correction. Elimination half-life of the 2 preparations showed there is no significant difference ($P>0.05$). However, t_{max} of solution preparation was shortened. The concentration of wogonin in plasma by i.v. route declined sharply (over 1/6000) by 2 h after dose administration. The parent drug cannot be detected after 12 h and mean residence time (MRT) of i.g. administration was obviously prolonged compared to the i.v. route. A 3-session crossover study evaluated pharmacokinetic parameters. The results for 3 treatments are depicted in Table 1, Figures 3, and 4, which showed the mean plasma concentration-time profile in 6 beagles after the 3 types of experiments.

Absolute bioavailability was determined by dividing the dose-normalized area under the plasma concentration-time curve (AUC) resulting from i.g. administration by that from i.v. injection, which was expressed as $(AUC_{ig} \times (Dose_{iv}) / (AUC_{iv} \times (Dose_{ig})) \times 100\%$. Result differences between native drug and solution preparation were rather amazing. The absolute bioavailability of crude drug was very low (about 0.6%), while the absolute bioavailability of SD was increased by nearly 7 times that of crude drug (about 4%), as shown in Table 2.

Detection of the free type of Wogonin and its main metabolites (wogonin 7-β-D-glucuronic (W-7-G) acid) after wogonin arginine solution agent gastric administration in beagles

To investigate the reasons for the low level of the drug in the body, further experiments were performed. Six beagles were used in the study on the absolute bioavailability of wogonin; 1 week later, the experiment was started. The drug was administered via the intragastric route (5 mg/kg); the blood collection time points were the same as the last experiment involving intragastric administration sustained for 36 h. Before the plasma sample treatment, 100 μl of β-glucuronidase was added to the blood sample [31] (1000 units/ml of 50 mM $NH_4H_2PO_4$ buffer solution [pH 5.0]), and vortexed in a 37°C thermostatic water bath for 24 h (60 rpm).

The concentration of free drug in the blood was also lower by solution irrigation administration in beagles. Similar to the SD irrigation, the concentration of drug increased rapidly, followed by a rapid decline after 1 h, maintained at a lower concentration (<5 ng/mL), and sustained for approximately 24 h. The concentration of the wogonin 7-β-D-glucuronic acid in blood was very high (>100 times the concentration of free

Table 1. Comparison of statistical moment parameters of pharmacokinetics of different routes of administration of Wogonin to Beagles.

Parameters	i.v. (5 mg/kg)	i.g. native drug (15 mg/kg)	i.g. SDs (5 mg/kg)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD ^{bd}
$AUC_{(0-t)}$ ug/L*h	629.7 $\pm$ 111.8	7.1 $\pm$ 2.0 ^b	21.0 $\pm$ 3.2 ^{ad}
$AUC_{(0-\infty)}$ ug/L*h	631.9 $\pm$ 112.8	10.8 $\pm$ 5.0 ^b	21.9 $\pm$ 4.6 ^{bc}
$t_{1/2z}$ h	5.9 $\pm$ 6.9	10.1 $\pm$ 8.9 ^a	5.1 $\pm$ 2.6 ^c
t_{max} h	-- $\pm$ --	0.7 $\pm$ 0.3 ^b	0.3 $\pm$ 0.2 ^{bd}
C_{max} ug/L	6838.7 $\pm$ 1322.1	2.5 $\pm$ 1.1 ^b	7.9 $\pm$ 3.3 ^{bd}
CL_z L/h/kg	8.1 $\pm$ 1.5	1681.8 $\pm$ 830.2 ^b	844.5 $\pm$ 507.1 ^{bd}
V_z ug/L	67.9 $\pm$ 73.4	18266.4 $\pm$ 9922.5 ^b	4975.5 $\pm$ 1898.9 ^{bd}
$MRT_{(0-t)}$ h	0.12 $\pm$ 0.03	3.6 $\pm$ 0.2 ^b	5.3 $\pm$ 3.0 ^{bd}

Compared with the iv group, ^a $p < 0.05$, ^b $p < 0.01$; Compared with the ig group, ^c $p < 0.05$, ^d $p < 0.01$.

Figure 3. Concentration-time curves of different forms of intravenous administration. **Figure 4.** Concentration-time curves of different forms of gastric administration (crude drug and solid dispersion).**Table 2.** Bioavailability of different routes of administration of Wogonin to beagles.

Number	i.v. (5 mg/kg)	i.g. native drug (15 mg/kg)	i.g. solid dispersions (5 mg/kg)	AB/%		RB/%
				i.g. native drug (15 mg/kg)	i.g. solid dispersions (5 mg/kg)	
	$AUC_{(0-\infty)}/ug/L*h$					
No1	479.6	16.7	31.8	1.2	6.6	571.3
No2	748.5	7.4	9.4	0.3	1.3	381.1
No3	698.0	9.5	17.0	0.5	2.4	536.8
No4	743.1	15.2	51.2	0.7	6.9	1010.5
No5	561.6	5.1	15.3	0.3	2.7	900.0
Mean	646.2	10.8	24.9	0.6	4.0	679.9
sd	119.8	5.0	16.8	0.4	2.6	264.3

Dog number 6 had vomiting after the administration of crude drug, which caused an inaccurate dosage of medication. The data of this dog was not included in the calculation of the bioavailability.

Figure 5. Beagles were administrated wogonin solution (5 mg/kg) via the intragastric route. Concentration-time curves of free wogonin and its main metabolite, wogonin- β -D-glucuronic (W-7-G) acid. Plasma concentrations of W-7-G in dogs are expressed as wogonin equivalents.

drugs), followed by a rapid decline that may be related to its rapid distribution to other tissues, and a sustained low concentration after 4 h (<20 ng), but could still be detected after 36 h (Figure 5).

The total blood concentration of free-type wogonin and β -glucuronidase -enzymolyzed wogonin was analyzed with the non-compartment model by medical professional statistical software (DAS2.0). The pharmacokinetic parameters of the main drugs are shown in Table 3. The free-type of wogonin in the blood was calculated, and the C_{max} of the solution agent administered via the intragastric route to beagles was higher than SD (12.3 ± 3.3 vs. 7.9 ± 3.3 $\mu\text{g/L}$), $\text{MRT}_{(0-t)}$ was comparatively shorter (3.39 ± 1.25 vs. 5.3 ± 3.0 h), and $\text{AUC}_{(0-\infty)}$ was decreased (17.8 ± 70.4 vs. 21.9 ± 16.8 $\mu\text{g/L} \cdot \text{h}$). No significant differences were found in other pharmacokinetic parameters. The absolute

Figure 6. Chemical structure of wogonin.

bioavailability of crude drug was very low (approximately 0.6%), while the absolute bioavailability of SD was increased by nearly 7 times compared to the crude drug (only 4%).

The relative bioavailability of the SD of wogonin was increased by approximately 679.9% compared with crude drug. To investigate the reasons for the low bioavailability of wogonin, LC/MSⁿ/DAD technology was used to analyze the plasma of beagles after drug administration. Glucose acid conjugates were confirmed as the main metabolite of wogonin and no other metabolite was found. Beagles were administered the salt solution of wogonin orally. The total concentration of the wogonin in the plasma of the free-type and the wogonin after hydrolysis was detected. The relative bioavailability of salt solution of wogonin was 81.3% compared with SD. The total wogonin after enzymolysis by β -glucuronidase was calculated. The C_{max} was approximately 12 times greater than the free-type of wogonin. $\text{AUC}_{(0-\infty)}$ was >30 times. $\text{MRT}_{(0-t)}$ was significantly prolonged (8.38 ± 3.14 vs. 3.39 ± 1.25 h).

Discussion

Wogonin, 5,7-dihydroxy-8-methoxyflavone (Figure 6), a major bioactive flavonoid aglycones isolated from the roots of

Table 3. Statistical moment parameters of pharmacokinetics of Wogonin gastric administration to beagles (5 mg/kg; calculation of the free Wogonin and total Wogonin after enzymolysis, respectively).

Parameters	Free Wogonin	Total Wogonin
	Mean $\pm$ SD	Mean $\pm$ SD
$\text{AUC}_{(0-t)}$ $\mu\text{g/L} \cdot \text{h}$	16.2 ± 6.0	449.2 ± 165.4
$\text{AUC}_{(0-\infty)}$ $\mu\text{g/L} \cdot \text{h}$	17.8 ± 7.4	554.8 ± 371.4
$t_{1/2z}$ h	4.94 ± 2.53	9.1 ± 5.2
T_{max} h	0.35 ± 0.21	0.51 ± 0.13
C_{max} $\mu\text{g/L}$	12.3 ± 3.3	156.5 ± 40.9
CL_z L/h/kg	969.8 ± 385.4	11.5 ± 4.8
V_z $\mu\text{g/L}$	6168.7 ± 2013.6	130.4 ± 40.1
$\text{MRT}_{(0-t)}$ h	3.39 ± 1.25	8.38 ± 3.14

Scutellaria baicalensis, was demonstrated to have many pharmacologic effects and biological activities, including anti-cancer [6], anti-viral [7], anti-oxidative [8], anti-inflammatory [9], and anti-seizure [10] effects. However, the water solubility of wogonin is very low, which limits its use. To improve the solubility and bioavailability of wogonin, researchers have used many kinds of processing technologies. Among various approaches, solid dispersion (SD) technology is promising in the application of low-solubility drugs [21–23], and the SD loaded with wogonin has not been reported previously.

In this study, the SDs were characterized by XRD and DSC analysis and showed that wogonin existed in an amorphous state in solid dispersions (SDs). In PM with a mount of the polymer (1:5 ratio), no diffraction peaks were discovered due to the polymer dilution effect [32,33]. The disappearance of characteristic peaks in all SDs suggested a significant decrease in wogonin crystallinity, demonstrating a drug amorphization or its solvation in the amorphous carriers [5,33,34]. DSC analysis enables the quantitative detection of all processes in which energy is required or produced. It allows exploring the process of melting, crystallization, evaporation, phase equilibriums, absorption, dehydration, sublimation, and substance degradation [35,36]. The lower intensity of the melting peak might be related to the dilution effect of polymer and, and the shift of the endothermic peak to lower temperature could be a result of dissolving wogonin in the melted PVP solution or the possible heat-induced interactions between the drug and polymer [37]. The study on solubility *in vitro* showed that the dissolution rate of wogonin-loaded solid dispersions prepared by PVP K30 was significantly increased compared with the physical mixture and the crude material. In addition to these mechanisms, an improvement in the dissolution rate of SDs could also be attributed to the drug particle size reduction and decrease in the drug crystallinity during the preparation of SDs [23,33,35]. The release rate enhancement could also be explained by the solubilizing and wetting effect of PVP K30 [33,36]. Increasing drug wettability and solubility as well as deaggregation of the drug particles brought about by the polymer could be the reasons for enhanced drug release rate from the SDs [14,21,32,33].

Until now, only limited data have been available on the pharmacokinetics of wogonin in rats. The HPLC method with the lower limit of quantitation (LLOQ) of 20 ng/mL lacked the sensitivity necessary for the pharmacokinetics study of wogonin [27]. In this report, we validated a simple and rapid LC-MS/MS method as being accurate, precise, and robust for determination of

wogonin in dog plasma. The main pharmacokinetic parameters were analyzed with the non-compartment model by medical professional statistical software (DAS2.0). The V_z of the 2 kinds of irrigation was much larger than the body fluid volume, which indicated that the drug concentration was distributed to a specific organ or tissue. The V_d of intravenous administration was small, which indicated that drug metabolism or excretion was faster by the intravenous administration and that prototype drug *in vivo* retention time is relatively short. To investigate the reasons for the low level of the drug in the body, further experiments were performed. In the current study, salt solution of wogonin was given to beagles by intragastric administration (5 mg/kg). The C_{max} of prototype drug was 12.3 ± 3.3 ng/mL and the average half-life was 4.94 ± 2.53 h. At 36 h after administration, no drug was detected. The C_{max} of wogonin after enzymolysis was significantly increased (156.5 ± 40.9) ng/mL. The average half-life was also prolonged to (9.1 ± 5.2) h. The individual time points after administration for 36 h were detected. Therefore, we deduced that the low bioavailability of wogonin might be caused by its low solubility associated with the fast binding with glucuronic acid *in vivo*. For most of the drugs, the phase II metabolites were not active *in vivo*. There were also several of the phase II drug metabolite activities that were stronger than the prototype drug. For example, the activity of the new cholesterol absorption inhibitor, ezetimibe, and morphine glucose acid conjugates was much stronger than the prototype [38].

Conclusions

The solvent method was successful using PVP K30 carrier for preparing SDs of wogonin. The solid state studies confirmed that solid dispersion of wogonin with carriers can decrease crystalline form of the drug or increase amorphousness of the drug, which can increase in the dissolution rate of wogonin from solid dispersion formulations. Further study indicates that low bioavailability of wogonin might be caused by its low solubility and associated with the fast binding with glucuronic acid *in vivo*. Research on the pharmacologic activity of wogonin glucuronic acid has important significance to the research and development of the drug.

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References:

- Sattar M, Sayed OM, Lane ME: Oral transmucosal drug delivery – current status and future prospects. *Int J Pharm*, 2014; 471(1–2): 498–506
- Lajoinie A, Henin E, Kassai B, Terry D: Solid oral forms availability in children: A cost saving investigation. *Br J Clin Pharmacol*, 2014; 78(5): 1080–89
- Sprunk A, Strachan CJ, Graf A: Rational formulation development and *in vitro* assessment of SMEDDS for oral delivery of poorly water soluble drugs. *Eur J Pharm Sci*, 2012; 46(5): 508–15
- Elder DP, Holm R, Diego HL: Use of pharmaceutical salts and cocrystals to address the issue of poor solubility. *Int J Pharm*, 2013; 453(1): 88–100
- Sarode AL, Malekar SA, Cote C, Worthen DR: Hydroxypropyl cellulose stabilizes amorphous solid dispersions of the poorly water soluble drug felodipine. *Carbohydr Polym*, 2014; 112: 512–19
- Li-Weber M: New therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer Treat Rev*, 2009; 35(1): 57–68
- Guo Q, Zhao L, You Q et al: Anti-hepatitis B virus activity of wogonin *in vitro* and *in vivo*. *Antiviral Res*, 2007; 74(1): 16–24
- Wang F, Song X, Zhou M et al: Wogonin inhibits H₂O₂-induced vascular permeability through suppressing the phosphorylation of caveolin-1. *Toxicology*, 2013; 305: 10–19
- Lee YM, Cheng PY, Chen SY et al: Wogonin suppresses arrhythmias, inflammatory responses, and apoptosis induced by myocardial ischemia/reperfusion in rats. *J Cardiovasc Pharmacol*, 2011; 58(2): 133–42
- Piao HZ, Choi IY, Park JS et al: Wogonin inhibits microglial cell migration via suppression of nuclear factor-kappa B activity. *Int Immunopharmacol*, 2008; 8(12): 1658–62
- Takagi R, Kawano M, Nakagome K et al: Wogonin attenuates ovalbumin antigen-induced neutrophilic airway inflammation by inhibiting th17 differentiation. *Int J Inflam*, 2014; 2014: 571508
- Lucas CD, Dorward DA, Sharma S et al: Wogonin induces eosinophil apoptosis and attenuates allergic airway inflammation. *Am J Respir Crit Care Med*, 2015; 191(6): 626–36
- Enomoto R, Suzuki C, Koshiba C et al: Wogonin prevents immunosuppressive action but not anti-inflammatory effect induced by glucocorticoid. *Ann NY Acad Sci*, 2007; 1095: 412–17
- Ramadhani N, Shabir M, McConville C: Preparation and characterisation of Kolliphor(R) P 188 and P 237 solid dispersion oral tablets containing the poorly water soluble drug disulfiram. *Int J Pharm*, 2014; 475(1–2): 514–22
- Taha EI, Ak-Suwayeh SA, Tayel MM, Badran MM: Fast ultra-fine self-nano-emulsifying drug delivery system for improving *in vitro* gastric dissolution of poor water soluble drug. *Acta Pol Pharm*, 2015; 72(1): 171–78
- Ahuja N, Katare OP, Singh B: Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water-soluble drug using water-soluble carriers. *Eur J Pharm Biopharm*, 2007; 65(1): 26–38
- Chen H, Khemtong C, Yang X et al: Nanonization strategies for poorly water-soluble drugs. *Drug Discov Today*, 2011; 16(7–8): 354–60
- Mallappa MK, Kesarla R, Banakar S: Calcium alginate-neusilin US2 nanocomposite microbeads for oral sustained drug delivery of poor water soluble drug aceclofenac sodium. *J Drug Deliv*, 2015; 2015: 826981
- Schram CJ, Beaudoin SP, Taylor LS: Impact of polymer conformation on the crystal growth inhibition of a poorly water-soluble drug in aqueous solution. *Langmuir*, 2015; 31(1): 171–79
- Aburahma MH: Bile salts-containing vesicles: Promising pharmaceutical carriers for oral delivery of poorly water-soluble drugs and peptide/protein-based therapeutics or vaccines. *Drug Deliv*, 2015 [Epub ahead of print]
- Konno H, Handa T, Alonzo DE, Taylor LS: Effect of polymer type on the dissolution profile of amorphous solid dispersions containing felodipine. *Eur J Pharm Biopharm*, 2008; 70(2): 493–99
- Nepal PR, Han HK, Choi HK: Enhancement of solubility and dissolution of coenzyme Q10 using solid dispersion formulation. *Int J Pharm*, 2010; 383(1–2): 147–53
- Sethia S, Squillante E: Solid dispersion of carbamazepine in PVP K30 by conventional solvent evaporation and supercritical methods. *Int J Pharm*, 2004; 272(1–2): 1–10
- Mishra DK, Dhote V, Bhargava A et al: Amorphous solid dispersion technique for improved drug delivery: Basics to clinical applications. *Drug Deliv Transl Res*, 2015; 5(6): 552–65
- Piao H, Yang L, Piao H et al: A pre-formulation study of a polymeric solid dispersion of paclitaxel prepared using a quasi-emulsion solvent diffusion method to improve the oral bioavailability in rats. *Drug Dev Ind Pharm*, 2016; 42(3): 353–63
- Newa M, Bhandari KH, Li DX et al: Preparation, characterization and *in vivo* evaluation of ibuprofen binary solid dispersions with poloxamer 188. *Int J Pharm*, 2007; 343(1–2): 228–37
- Xu Y, Ke X, Ping QN: Wogonin pharmacokinetic behavior in rats after intravenous injection. *Pharmaceutical and Clinical Research*, 2007; 15(01): 35–38
- Tsai TH, Chou CJ, Tsai TR, Chen CF: Determination of wogonin in rat plasma by liquid chromatography and its pharmacokinetic application. *Planta Med*, 1996; 62(3): 263–66
- Emami S, Valizadeh H, Islambulchilar Z, Zakeri-Milani P: Development and physicochemical characterization of sirolimus solid dispersions prepared by solvent evaporation method. *Adv Pharm Bull*, 2014; 4(4): 369–74
- Chen XQ, Stefanski K, Shen H et al: Oral delivery of highly lipophilic poorly water-soluble drugs: Spray-dried dispersions to improve oral absorption and enable high-dose toxicology studies of a P2Y1 antagonist. *J Pharm Sci*, 2014; 103(12): 3924–31
- Chen X, Wang H, Du Y, Zhong D: Quantitation of the flavonoid wogonin and its major metabolite wogonin-7 beta-D-glucuronide in rat plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2002; 775(2): 169–78
- Siahi-Shadbad MR, Ghanbarzadeh S, Barzegar-Jalali M et al: Development and characterization of solid dispersion for dissolution improvement of furosemide by cogrinding method. *Adv Pharm Bull*, 2014; 4(4): 391–99
- Jahangiri A, Barzegar-Jalali M, Garjani A et al: Physicochemical characterization and pharmacological evaluation of ezetimibe-PVP K30 solid dispersions in hyperlipidemic rats. *Colloids Surf B Biointerfaces*, 2015; 134: 423–30
- Soti PL, Bocz K, Pataki H et al: Comparison of spray drying, electroblowing and electrospinning for preparation of Eudragit E and itraconazole solid dispersions. *Int J Pharm*, 2015; 494(1): 23–30
- Kim EJ, Chun MK, Jang JS et al: Preparation of a solid dispersion of felodipine using a solvent wetting method. *Eur J Pharm Biopharm*, 2006; 64(2): 200–5
- Ruan LP, Yu BY, Fu GM, Zhu DN: Improving the solubility of amlopipin by solid dispersions and inclusion complexes. *J Pharm Biomed Anal*, 2005; 38(3): 457–64
- Barzegar-Jalali M, Ghanbarzadeh S, Adibkia K et al: Development and characterization of solid dispersion of piroxicam for improvement of dissolution rate using hydrophilic carriers. *Bioimpacts*, 2014; 4(3): 141–48
- Kosoglou T, Statkevich P, Johnson-Levonas AO et al: Ezetimibe: A review of its metabolism, pharmacokinetics and drug interactions. *Clin Pharmacokinet*, 2005; 44(5): 467–94