广 西 植 物 Guihaia 32(3):392－399 2012 年 5 月

DOI: 10.3969/j.issn.1000-3142.2012.05.022

Pre-clinical pharmacokinetics of novel soybean iosflavone sulfonate

PENG You[1,2*], TAO Chun-Yuan[1], DENG Ze-Yuan[2]

(1. *Department of Chemistry and Engineering, Jiujiang University*, Jiujiang 332005, China; 2. *State Key Laboratory of Food Science and Technology, Nanchang University*, Nanchang 330047, China)

Abstract: The oral bioavailability of genistein (GE) in its benzensulfonate prodrug was studied in search for its novel prodrug. The plasmas were collected at different points of time after the intragastric or intravenous administration of genistein benzensulfonate (GBS) 40 mg/kg to rats. The GBS and GE contents in plasma were determined by HPLC. The compartment model was fitted and pharma cokinetic parameters were calculated by DAS 2.1.1. The results indicated that the dynamic processes of GE were consistent with two compartment model after intragastric administration of GBS prodrug to rats. The relative oral bioavailability of GE in prodrug GBS was 198.6%. In conclusion, the above results demonstrated that the oral bioavailability of GE in prodrug had been improved remarkably.

Key words: genistein sulfonate; prodrug; pharmacokinetics; bioavailability

CLC Number: R965.1 **Document Code**: A **Article ID**: 1000-3142(2012)03-0392-08

新型大豆异黄酮磺酸酯的临床前药物动力学

彭 游[1,2*] 陶春元[1], 邓泽元[2]

(1. 九江学院 化工学院, 江西 九江 332005; 2. 南昌大学 食品科学与技术国家重点实验室, 南昌 330047)

摘 要: 为寻找新的大豆异黄酮前药,采用建立的生物样品中药物浓度测定的液相色谱法对新型大豆异黄酮染料木素磺酸酯(GBS)进行前药判定以及大鼠体内药物动力学研究,以考察前药中染料木素(GE)的口服相对生物利用度是否改善。在大鼠体内药物代谢实验中,灌胃给予的大鼠血浆中能检测到 GE 的存在。在临床前药物动力学实验中,该前体药以 40 mg/kg GE 在大鼠体内的动力学过程符合一室模型。GBS 中 GE 的相对口服生物利用度为原药的 198.6 %。结果表明:相对于原药 GE,前药中 GE 的相对口服生物利用度得到极大地改善。该前药有进一步研究的意义。

关键词: 染料木素磺酸酯; 前药; 药物动力学; 生物利用度

1 Introduction

Soybean isoflavone is a class of secondary metabolite in soybean(Peng *et al.*,2011). In recent years the abundant researches indicated the soybean isoflavones in food had many important physiological functions including preventing cancer, cardiovascular disease and the osteoporosis sickness, anti-oxidation, reducing the female menopause syndromea and the blood sugar, anti-senilly, and so on(Coward etal,1993; Aedin *et al.*, 2000; Holder *et al.*,1998; Huang *et al.*,2004). However, among the isoflavones, GE includes three polar group hydroxyl, its lipophilicity was weak. Simultane-

* **Received date**: 2011-09-05 **Accepted date**: 2011-12-23

Foundation items: Supported by the National Natural Science Foundation of China(81160412); the Natural Science Foundation of Jiangxi Province (2010GZN0106); the Science and Technology Item of the Education Department of Jiangxi Province(GJJ11626)

Biography: PENG You(1971-), male, Doctor Degree, Associate Professor, Working With Natural Product Chemistry Research, (E-mail) trihydracid@126.com.

* Author for Correspondence

ously its hydroxyls formed the intermolecular hydrogen bond, its lattice energy was high, so its hydrophility was not strong, too. Only a small part of GE could be absorbed rapidly and metabolized to glucuronic acid glycosides in vivo, most were degraded and metabolized by microorganisms in the intestine. So, GE had the first pass metabolism in the intestine (Barne *et al.*, 1996). Thus, the defect of solubility and the first pass effect caused its low bioavailability and biological activities. Until now it cannot be used widespread effectively in clinical (Andlauer *et al.*, 2000).

To improve the oral bioavailability and pharmacological action of GE, our laboratory had synthesized GBS by the prodrug principle (Peng *et al.*, 2008). We

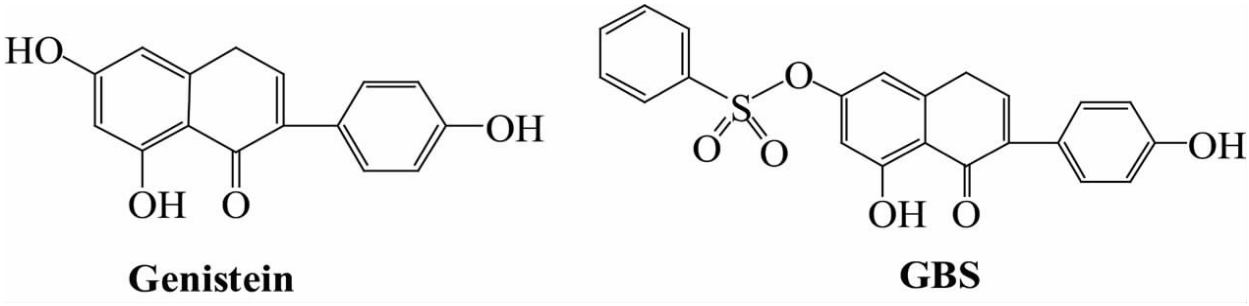

Fig. 1　Structure of genistein and their derivatives

hope these compounds could radically improve solubilities and metabolic stabilities, increase its in vivo bioavailabilities and anti-cancer activities (Fig. 1). To isolate new prodrug that has a good affinity and high bioavailability, metabolisms and pharmacokinetics of GBS were studied in animals.

Taking daidzein (DZ) as an internal standard, simultaneous determination of GE and GBS in biological samples were found by HPLC method, which was also used to study in vivo metabolism and pharmacokinetics of GE and GBS.

2　Materials and methods

2.1 Reagents and materials

Chemicals GBS was synthesized by our lab (the purity, greater than 99%, HPLC determination). DZ and GE were provided by Shanxi Huike Botanical Development Co., Ltd. High performance liquid chromatography (HPLC)-grade methanol (produced by Merck Corp.) were used. PEG-400, formic acid, methanol etc were of analytical grade. Animals Fifteen of Wistar rats with female/male (2/3) at 200—250 g of body weight, were provided by the Experimental Animal Center of Nanchang University (Certificate of Conformity: SCXK-2009-0009).

2.2 Assay

Concentrations of drugs were determined in plasma using a RP-HPLC method. Analysis was performed on an Agilent 1 100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, a vacuum degasser, an automatic injector, and a variable wavelength detector. Separation was carried on a 5 μL Venusil XBP-C_{18} column (250 mm×4.6 mm, Agela Technologies, China) using 0.1% formic acid aqueous solution: methanol (1 ∶ 1, v/v) as the mobile phase at a flow rate of 0.5 mL/min. The effluent was monitored at 262 nm with detector sensitivity of 2.00 AUFS, and the maximum absorbance waves for GBS was determined at 262 nm with a DAD detector. Agilent Chromatographic Station was used. The column temperature was kept at 20 ℃. The injected volume of the analyzed samples was 10 μL.

2.3 Plasma sample pretreatment

Following addition of 10 μL DZ solution (100 μg/mL) to 40 μL of plasma sample, the sample was swirled for 3 min. And then, 1.0 mL of ethyl acetate was added to this sample. The sample was swirled for 3 min again and centrifuged at 14 000 r/min and 4 ℃ for 10 min to separate layers. The organic layer was transferred to clean tube and evaporated to dryness under nitrogen. The residue was

dissolved in 200 μL of methanol, and 10 μL was injected into the HPLC system.

2.4 Solution preparation

2.4.1 Administration of solution in drug metabolism Taking minutely 50 mg of drug, added 0.1—0.2 mL dimethyl sulfoxide(DMSO), dissolved and diluted to 5.0 mL with PEG-400, so a concentration of 10 mg/mL solution of the drug was prepared for oral administration of rats in the prodrug screening experiments. The solution was prepared and used presently.

2.4.2 Administration of solution in pharmacokinetics Taking minutely 40 mg of GE(GBS), added 0.1—0.2 mL DMSO, dissolved and diluted to 8.0 mL with PEG-400, so a concentration of 5 mg/mL solution of the drug was prepared for oral administration of rats. Taking minutely 40 mg of GBS, added 0.1—0.2 mL DMSO, dissolved and diluted to 2.0 mL with PEG-400, so a 20 mg/mL solution of the prodrug was prepared for intravenous administration of rats. The solution was prepared and used presently.

2.5 The drug metabolism research in vivo

2.5.1 Intragastric administration Healthy Wistar rats with weight 200—250 g were fasted 12 h and given access to water freely before administration. The drug GBS was gavaged by 100 mg/kg dose to a rat. At 5, 10, 30 min and 1 h after administration, 0.1—0.2 mL of blood was collected through the orbital venous plexus respectively. After centrifuged chilled 10 min with 14 000 r/min, blood was separated to plasma. Plasma sample was preserved at the -70 ℃ until pretreatment.

2.5.2 Plasma sample preparation Combining of 0—1 h plasma samples, to 0.5 mL of the sample was added 50 μL of methanol. The rear operation accorded "2.3 Plasma sample pretreatment" method.

2.6 The pharmacokinetics in rats

2.6.1 Dosage regimen in pharmacokinetic experiments and sample collection Intragastric administration Ten healthy Wistar rats, female/male(2/3), weight 200—250 g, were randomly divided into 2 treatment groups. All animals were fasted 12 h and accessed to water freely before administration. The drug was gavaged by 40 mg/kg dose (dose volume of 10 mL/kg) respectively. At before administration(0 h), 0.08, 0.17, 0.33, 0.67, 1, 2, 4, 6, 8, 12, 24 h after administration, 0.1 mL of blood was collected through the orbital venous plexus to heparin tube respectively. After centrifuged chilled at 14 000 r/min for 10 min, the upper layer blood plasma sample was preserved at the -70 ℃ until analysis.

Intravenous administiation Randomly five healthy Wistar rats, female/male(2/3), weight 200—250 g, were divided into a treatment group. All animals were fasted 12 h and accessed to water freely before administration. Respectively the prodrug was injected via the tail vein by 40 mg/kg dose(dose volume of 2 mL/kg). At before administration(0 h), 0.08, 0.17, 0.33, 0.67, 1, 2, 4, 6, 8, 12, 24 h after administration, 0.1 mL of blood was collected through the orbital venous plexus to heparin tube respectively. After centrifuged chilled at 14 000 r/min for 10 min, the upper layer blood plasma sample was preserved at the -70 ℃ until analysis.

2.6.2 Determination of drug concentration Flowing oral or intravenous administration of drugs, the concentrations of the original medicine and the precursor in plasma sample were determined separately by the HPLC method which had been established.

2.6.3 Data analysis Under the standard curve established by each analysis group, drug concentration was calculated in plasma. Using pharmacokinetic calculation software DAS 2.1.1, the main pharmacokinetic parameters of drugs were calculated through the non-compartment model. The experimental data were statistically analyzed through test. If $P<0.05$, considered significant difference, $P<0.01$ considered highly significant difference and $P>0.05$ no significant difference.

3 Results and analysis

3.1 Analysis confirmed

3.1.1 Method selectivity The rat blank plasma 50 μL were treated according to "2.3 Blood sample pretreatment" method(without internal standard). 10 μL was injected into HPLC system and chromatogram Fig. 2(A) was obtained. The standard solution of the lower limit of quantification of GE, GBS was added into

a blank plasma respectively and operated according to the same method(with internal standard), so chromatogram Fig. 2(B) was obtained, in which the retention time of GE, GBS, and DZ was 13.4, 16.2, 12.4 min respectively. The results showed that endogenous substances in plasma did not interfere with the determination of GE, GBS and DZ, also the internal standard DZ and the tested samples did not interfere with each other. The analytic period of each sample was 26.5 min.

3.1.2 Preparation of standard curve　Adding 10 μL of GE standard series solution to 40 μL of rat blank plasma and eddying 3 min, so their concentration(μg/mL) were 0.02, 0.08, 0.25, 1.0, 2.5, 10, 40 respectively. The other operation accorded to "2.3 Blood sample pretreatment" method. To the concentration of analytes as abscissa, the ratio of peak area of analytes and internal standard as the vertical axis, carried on the return operation with the weighting least squares method, the standard curve linear regression equation obtained was $Y=2\times10^{-5}X+0.0084$, $R^2=0.9997$. The linear range of the determination of concentration of GE in plasma was 0.020－40 μg/mL according to the standard curve. The preparation of standard curve of GBS accorded with GE in plasma.

The straight linear regression equation of standard curve of GBS in plasma: $Y=1\times10^{-4}X+0.0654$, $R^2=0.9994$, the linear range was 0.025－32 μg/mL.

Adding 10 μL of standard solution of GE to 40 μL of blank plasma, the sample of the equivalent with GE 0.02 μg/mL was obtained. Carried on 5-sample analysis to the sample, based on the standard curve of the same day, the concentration of each sample was obtained. The results showed that the lower limits of quantification of GE and GBS in plasma were 0.02 μg/mL and 0.025 μg/mL with HPLC method respectively. The with-day precision(relative standard deviation, RSD) of GE and GBS in lower limit of quantification concentration were 13.7% and 11.9% respectively, and their accuracies (RE) in this concentration were 9.2% and -3.2% respectively.

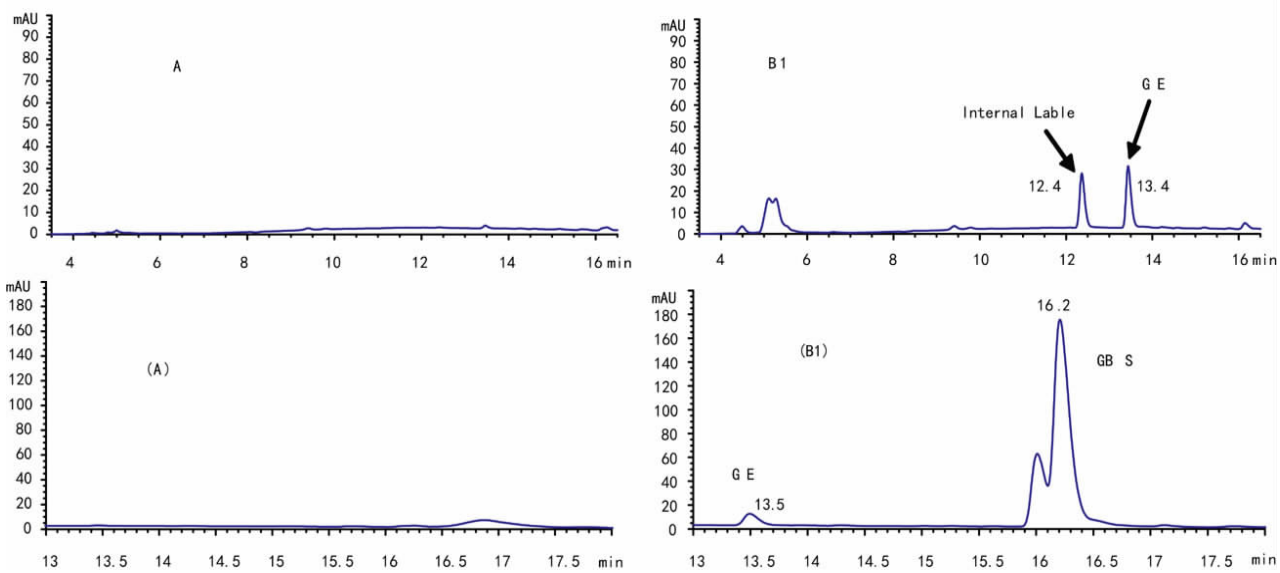

Fig. 2　Representative chromatograms of genistein and its derivatives in plasma samples determined by HPLC method (**A**) A blank plasma sample; (**B**) A blank plasma sample spiked with GE(**B1**), GBS(**B2**) at the lower limit of quantification.

3.1.3 The precision and accuracy of method　Taking 40 μL blank plasma, the low, medium and high concentrations of three quality control(QC) samples of GE were prepared according to "Preparation of standard Curve" method. Each concentration was completed for 6-sample analysis and determined for three days continuously, and their standard curves were prepared at the same time. Calculating the concentration of QC samples and comparing with the added concentration, the accuracy and precision of determination method of each component was sought. The determination method of RE and RSD of GBS accorded with the determi-

nation of GE. Experimental data indicate that the with-day and between-day precision of GE, GBS were less than 12%, and accuracy were at ±13%. The determination method of compounds tested in plasma was in compliance with the relevant international standards requirements(Shah *et al*., 2000).

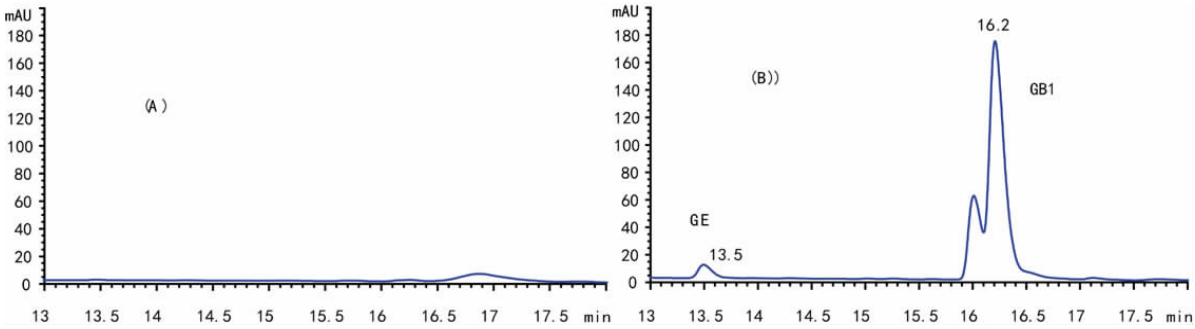

Fig. 3 HPLC of compounds in rat plasma after oral administration of 100 mg/kg derivative GBS to Wistar rats. A-blank plasma

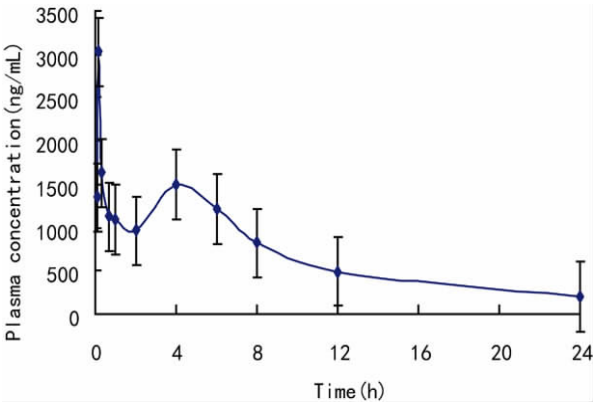

Fig. 4 The mean plasma concentration-time curve of genistein following oral administration of 40 mg/kg genistein to Wistar rats(n=5).

Each value represents the mean±SD of five individual values

3.1.4 The extraction recovery of sample According to"3.1.2 Prepation of standard Curve"method, the QC samples of GE, GBS and DZ were prepared and the extraction recovery of these samples were reviewed. The results showed that the extraction recovery of three kinds of concentrations of the components were over 70% in general, only the recovery of GE was 66.1% at high concentration.

3.1.5 The stability of sample According to"3.1.2 Preparation of standard Curve"method, the low, medium and high concentrations of three QC samples of GE, GBS were prepared to study the stability of samples in three different preservation conditions. The results showed that the tested compounds were stable at the -70 ℃ during 30 days, as well as after three freezing-defrosting circulation. The plasma sample solutions extracted were stable after 24 h at room temperature. The RE of various concentration groups were —7.8%—12.8%。

3.2 The drug metabolism research in vivo

The synthetic compound was ester of GE. The prodrug theory believed that only the derivative could be degradated and release parent drug timely in vivo that play a pharmacological effect, so it would be carried out its metabolic studies in vivo to confirm whether it was prodrug. After the Wistar rat was being oral administration of 100 mg/kg GBS, its plasma samples were analysised using HPLC. The experimental results in Fig. 3 showed that GE could be detected in rat plasma obviously after oral administration of GBS to Wistar rats. Thus GBS was the prodrug of GE. From Fig. 3(B), it was obvious that GBS could also be detected in rat plasma after oral administration of GBS.

3.3 Pharmacokinetics

3.3.1 Pharmacokinetics of the parent drug in rats

Pharmacokinetics of parent drug GE in rats in vivo was studied in detail in the literature(Setchell *et al*., 2003). To accurately investigate its relative bioavailability in GBS, its pharmacokinetics after its intragastric administration to rats under the same experimental conditions was also studied.

Following oral administration with 40 mg/kg GE to rats, its mean plasma concentration - time curve see Fig. 4. The blood drug concentration data were fitted

with compartment model and pharmacokinetic parameters were calculated by the DAS 2.1.1. It found the dynamic processes of GE consistent with a compartment model after intragastric administration of GE to rats. The pharmacokinetic parameters respectively were: $t_{1/2}$, (8.18±5.30) h; C_{max}, (3511±2 408) ng/mL; T_{max}, (1.20±1.60) h; AUC_{0-t}, (15 550±1 649) ng.h/mL; $AUC_{0-\infty}$, (18 665±2 879) ng.h/mL.

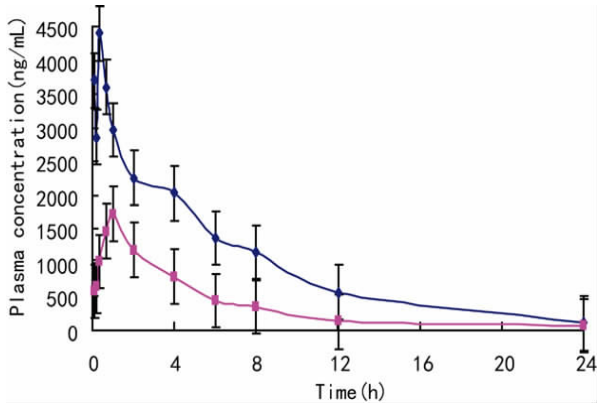

Fig. 5　The mean plasma concentration-time curves of GE(◆) and GBS(■) following oral administration of 40 mg/kg GBS to to Wistar rats(n=5).
Each value represents the mean±SD of five individual values.

3.3.2 Pharmacokinetics of prodrug GBS　The pharmacokinetic behaviors were studied after intragastric or intravenous administration of GBS to rats with established HPLC method. After oral and intravenous administration with 40mg/kg dose of GBS to rats, the mean plasma concentration-time curves of GE and GBS see Fig. 5 and Fig. 6 respectively. Blood drug concentration data were fitted with compartment model by the DAS 2.1.1 software and pharmacokinetic parameters were calculated. It found that the dynamic processes of GE consistent with a compartment model after intragastric administration of GBS to rats and that the dynamic processes of GE and GBS were all consistent with two-compartment model after intravenous administration of GBS to rats. Pharmacokinetic parameters see Table 1.

By the drug concentration-time curves of Fig. 5 and Table 1, it could be found that the T_{max} of GE was about 0.3 h after orally administration of GBS to rats. It indicated that the prodrug GBS was rapidly absorbed in vivo and hydrolyzed by enzymes to the parent drug GE. The half-life of GBS was 12.61 h, which indicated that GBS could be maintained a longer time in the blood, and also that is conducived to the maintenance of GE in the blood a long time.

The pharmacokinetic behaviors were studied after intragastric or intravenous administration of GBS to rats with established HPLC method. Following oral and intravenous administration of 40 mg/kg GBS to rats, the mean plasma concentration-time curves of GE and GBS see Fig. 5 and Fig. 6 respectively. The blood drug concentration data were fitted with compartment model and pharmacokinetic parameters were calculated by the DAS 2.1.1. It found that the dynamic processes of GE were all consistent with two compartment model after intragastric or intravenous administration of GBS to rats. Pharmacokinetic parameters see Table 1.

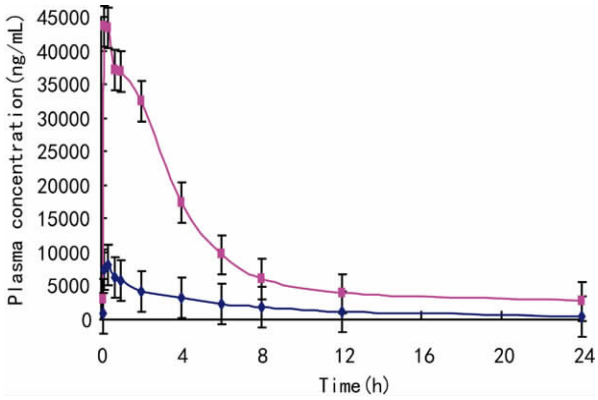

Fig. 6 The mean plasma concentration-time curves of GE(◆) and GBS(■) following intravenous administration of 40 mg/kg GBS to Wistar rats(n=5)
Each value represents the mean±SD of five individual values

By the drug concentration-time curves of Fig. 5 and Table 1, it could be found that the T_{max} of GE was about 0.53 h and the prodrug GBS was not detected in plasma after oral administration of GBS to rats. It indicated that the prodrug GBS was absorbed in vivo and hydrolyzed by enzymes to the parent drug GE rapidly. GE presented the twin peaks following oral administration of GBS to rats, the t_{max1}, t_{max2} of two peaks of GE were 40 min and 8 h respectively, and the C_{max1} and C_{max2} were 2 684 and 684 ng/mL respectively. GBS presented the twin peaks following intravenous administration of GBS to rats, the t_{max1}, t_{max2} of two peaks of

GBS were 5 min and 6 h respectively, and the C_{max1} and C_{max2} were 43 481 and 3 325 ng/mL respectively.

3.4 Pharmacokinetic characteristics of genistein prodrugs in rats

Fllowing intragastric administration of prodrugs, the T_{max}, $t_{1/2}$ and $AUC_{0-\infty}$ of GE that come from hydrolysis of prodrug GBS see Table 1. The dose of prodrugs converted into the equivalent of the same dose(D_{test}) of GE by equimolar respectively. The relative bioavailability(Fr) of GE in prodrugs was calculated by the formula(1), see Table 2.

$$Fr=[(D_{standard}\times AUC_{test})/(D_{test}\times AUC_{standard})]\times 100\%^{*} \quad (1)$$

*: $D_{standard}$: the oral dose of the parent drug; D_{test}: the dose of the parent drug converted by oral prodrug; $AUC_{standard}$: the area under the concentration-time curve of parent drug flowing oral administration of parent drug; AUC_{test}: the area under the concentration-time curve of parent drug flowing oral administration of prodrug.

Table 1 The mean non-compartmental pharmacokinetic parameters of genistein and GBS in Wistar rats after single oral and intravenous administration of 40 mg/kg GBS(n=5). Each valuerepresents the mean±SD for five individual values.

Parameters	Oral		Intravenous	
	Genistein	GBS	Genistein	GBS
t1/2(h)	4.41±1.07	12.61±10.61	7.20±1.09	5.56±2.50
T_{max}(h)	0.30±0.24	2.13±1.71		
C_{max}(ng/mL)	6548±2268	2050±797		
AUC_{0-t}(ng. h/mL)	22747±3611	8820±2005	44384±28278	221365±116160
$AUC_{0-\infty}$(ng. h/mL)	24411±3282	10678±3311	49378±31383	229320±110316

Table 2 The relative bioavailability of genistein and its prodrugs in Wistar rats after single oral administration

Parameter	GE	GE/GBS
$t_{1/2}$(h)	8.18±5.30	4.41±1.07
T_{max}(h)	1.20±1.60	0.30±0.24
$AUC_{0-\infty}$(ng. h/mL)	18665±2879	24411±3282
Fr (%)	—	198.6

After intragastric administration of prodrug, the T_{max} of GE from prodrug that was in one hour, was shorter than T_{max} of parent GE. Perhaps due to the large molecular weight, afer intravenous administration of the prodrug GBS, it occured hepatobiliary circulation.

According to the relatived bioavailability, prodrug in rats released the amount of GE in the order for the GBS>>GE. It indicated that the kinetic behavior of the prodrug GBS compared with GE was known as a great improvement by proper structural modification.

After respectively intragastric administration of the prodrug, the T_{max} of GE that came from hydrolysis of the prodrug in 1 h, and was shorter than T_{max} (1.2 h) of parent GE. After intragastric administration, the half-live was 4.41 h. From Fig. 1, compared to the GE, 7-OH of prodrug GBS was substituted by benzene sulfonate group. Its lipophilic was enhanced, and it might be better to avoid the first pass effect. Its relative bioavailability was larger than GE in expermients. GBS could be more likely to have further research value.

4 Discussion

The objective of design and synthesis of prodrugs was to solve the specific problems in pharmacy or pharmacology and give the useful nature to drugs. These mainly included: transformation their in vivo pharmacokinetics, adjustment in vivo absorption and distribution, improvement the stability and water solubility of drugs and so on. The main aim of sulfonic acid ester-modified of genistein was to improve its absorption in vivo and block first-pass effect, thus to enhance its bioavailability and pharmacological effects possibly. The prodrug design ideas of the polyhydroxy flavonoids were explored with the results of this experiments.

Polyhydroxy flavonoids, mainly referring to natu-

ral active product genistein, daidzein, quercetin, etc., have good chemical stability, no toxicity and human adverse reactions, but they have different degree of pharmacokinetics shortcomings, mainly the first-pass effects, intestinal flora decomposition, fast elimination in vivo, etc., and the same time also poor water solubility in general (Hur *et al*., 2000; Hollman & Katan, 1998). The deficiencies in pharmacokinetics and poor water solubility were main reasons for their low bioavailability. So prodrug modifition of such polyhydroxy flavonoids must start from improving these two areas weaknesses.

According to affecting factors of the drug bioavailability, combined with the physical and chemical properties of polyhydroxy flavonoids, as well as their basic transmission characteristic in vivo, the prodrug design for multi-hydroxyflavone was carried out mainly in lipophilic aspect. The big sulphonate group introduced in molecular seals up its easily metabolized phenol hydroxyl, improved its solubility and amphipathicity, enabled to have the good biomembrane endophilicity, increased the absorption rate, avoided the metabolism before circulation, enhanced the bioavailability.

This paper had carried on prodrug decision to the new sulphonate derivative of genistein, and conducted the of the prodrug confirmed. The experiments indicated that the bioavailability of genistein flowing oral administration of GBS was better than that of genistein following oral administration of genistein. This explained that thinking of optimizing kinetic property of genistein by sulphonate modification to enhance the oral bioavailability was successful.

5　Summary

The novel sulfonic acid ester derivative of genistein was its prodrug confirmed by metabolism screening studies in rats. Its pharmacokinetic studies have shown that the prodrug GBS have good oral bioavailability. The idea that we adopted the structural modification of the hydroxyl group of genistein to optimize the pharmacokinetic properties, block first-pass metabolism and thus has improved the oral bioavailability is feasible.

References:

Aedin C, Bryn H, Rosa M. 2000. Isoflavones, lignans and stilbenes-origins, metabolism and potential importance to human health[J]. *J Sci Food Agric*, **80**: 1 044－1 047

Andlauer W, Kolb J, Stehle P, *et al*. 2000. Absorption and metabolism of genistein in isolated rat small intestine[J]. *J Nutr*, **130**: 834－846

Barne S, Sfakinos J, Coward L. *et al*. 1996. Soy isoflavonoids and cancer prevention. Underlying biochemical and pharmacological issues[J]. *Adv Exp Med Biol*, **401**: 87－100

Coward L, Barnes NC, Setchell KDR, *et al*. 1993. Genistein, daidzein, and their β-glcoside conjugates: antitumor isoflavones in soybean foods from American and asian diets[J]. *J Agric Food Chem*, **41**: 1 961－1 967

Holder CL, Churchwell MI, Doerge DR. 1999. Quantification of soy isoflavones, genistein and daidzein, and conjugates in rat blood using LC/ES-MS[J]. *Agric Food Chem*, **47**: 3 764－3 770

Hollman PC, Katan MB. 1998. Bioavailability and health effects of dietary flavonols in man[J]. *Arch Toxicol Suppl*, **20**: 237－248

Huang J, Luo Q, Li XL, *et al*. 2004. Hyperglycemia lowering effect of soybean isoflavone(SI) on alloxan-induced diabetic mice [J]. *Food Sci*, **25**(1): 166－170 [Chinese]

Hur HG, Lay JO, Jr Beger RD, *et al*. 2000, Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genistin[J]. *Arch Microbiol*, **174**(6): 422－428

Peng Y, Deng ZY, Ye ZG, *et al*. 2011. Extraction of soybean isoflavone by microwave radiation without solvent and its mechanism[J]. *Guihaia*, **31**(2): 266－269

Peng Y, Deng ZY, Lang SJ, *et al*. 2008. Preparation and crystal structure of genistein benzensulfonate prodrugs[J]. *J Chem Res*, **10**: 555－558

Setchell KD, Faughnan MS, Avades T, *et al*. 2003. Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women[J]. *Am J Clin Nutr*, **77**: 411－419

Shah VP, Midha KK, Findlay JW, *et al*. 2000. Bioanalytical method validationa revisit with a decade of progress[J]. *Pharm Res*, **17**: 1 551－1 557