DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 10, pp. 1079–1087, 2004

Preparation and Evaluation of pH-Dependent Gradient-Release Pellets for TCM

Xing Tang,* XiaoLin Tian, and Lei Ci

School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, P.R. China

ABSTRACT

This paper is designed to investigate a novel sustained release system for Traditional Chinese Medicinal Compound Recipe (TCMCR) by incorporating three kinds of pH-dependent gradient-release coated pellets into capsules. In our study, dosage reform was conducted on the TCMCR model drug–Guanxin Suhe Wan (GSW), which is in the traditional form of honey bolus, comprising *Styrax*, *Borneolumsyntheticum*, *Olbanum*, *Radix aristolochiae* and *Lignum santali albi*. In this study, the β-CD inclusion complexes were prepared separately for *Styrax*, *Borneolumsyntheticum* and the volatile oil extracted from the mixture of *Olbanum*, *Radix aristolochiae* and *Lignum santali albi*. Pellets were prepared in a centrifugal granulator using the powder layering technique and then divided into 3 equal weight portions and coated with HPMC, HPMCP HP-55 and Eudragit L100/S100 to obtain gradient release in stomach, duodenum and jejunum or ileum respectively. On this basis, a pH-dependent sustained-release pellets system, ''Guanxin Suhe Sustained-release Capsules''(GSSC), was prepared by mixing the above three kinds of coated pellets at the weight ratio of 1:1:1. Pharmacokinetic (PK) studies between GSW and GSSC were made on male volunteers and isolated guinea pig hearts by plasma drug concentration method and serum pharmacology method respectively. In plasma drug concentration method, T_{max} was 0.42 h and 1.08 h for GSW and GSSC respectively, while in the serum pharmacology method, T_{max} was 0.56 h and 0.52 h respectively. The relative bioavailability of GSSC to GSW was 95.62% and 121.82% separately in the above two methods, indicating a similarity between the two methods in predicting the PK behavior of GSSC.

*Correspondence: Xing Tang, School of Pharmacy, Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenyang 110016, P.R. China; E-mail: tangpharm@sina.com.

DOI: 10.1081/DDC-200040286
0363-9045 (Print); 1520-5762 (Online)

INTRODUCTION

In China, medicinal plants and their preparations have been used for thousands of years. They can be adapted to various ailments in the forms of Traditional Chinese Medicine (TCM). In recent years, TCM is becoming so much in vogue because of the consumers' anxiety for the undesired secondary effects of synthetic drugs and the ''Green Movement'' which has been resurgent in Europe, North America and Australia.[1–5] Most of the TCM preparations are in the forms of pills, powders, soft extracts and others manufactured by traditional technological procedures.[6] However, these traditional technologies cannot meet the requirements of modernization, and many TCM preparations lack corresponding quality control standards. Therefore the development of new TCM delivery systems such as sustained-release system is a pressing task.[7,8] Traditionally, material medica sinica has been used in China in the form of compound prescriptions, most of which are formed not by simply putting together various ingredients, but by arranging them according to certain Chinese medical theories and principles of combination. A typical TCMCR is usually composed of principal components, deputy components, adjuvant components and the guide components. The principal component is a substance that provides the main therapeutic effects, while the deputy, adjuvant and guide components assist the therapeutic actions of the principal components.[9,10] When combined properly, the biologically active substances may have the mutual effects in terms of accentuation, enhancement, counteraction and suppression, etc.

In our study, Guanxin Suhe Wan (GSW) was selected as a TCMCR model drug. Listed in the first section of the Pharmacopoeia of the People's Republic of China (Ch.P, 2000), Guanxin Suhe Wan consists of *Styrax*, *Borneolumsyntheticum*, *Olbanum*, *Radix aristolochiae* and *Lignum santali albi*, in which *Styrax* is the principal component. In the clinical practice, GSW has long been primarily applied to the treatment of angina pectoris and myocardial infarction caused by coronary heart disease and has produced quite favorable effects.

The traditional dosage form of GSW is honey bolus, produced by mixing powders of the four crude drugs except *styrax* and then made into bolus with honey and *styrax*.[11,12] According to the research of chronopharmacology, there is an increased risk of myocardial infarction, angina pectoris, and sudden cardiac death in the early-morning hours.[13] For the prevention and treatment of coronary heart disease, a certain drug level must be maintained around early-morning hours. Thus the objective of the present study is to make a dosage reform by preparing Guanxin Suhe Sustained-release Capsules(GSSC) out of the same original crude drugs and formula ratio of GSW. The in vitro release and pharmacokinetic characteristics of the new preparation were also investigated.

MATERIALS AND METHODS

Materials

Styrax, *Borneolumsyntheticum*, *Olbanum*, *Radix aristolochiae* and *Lignum santali albi* were purchased from a drugstore in Shenyang (China). GSW was obtained from Shenyang Northeast Pharmaceutical Group. Cinnamic acid was ordered from the National Institute for the Control of Pharmaceutical and Biological Products. Microcrystalline Cellulose (MCC) was obtained from Changshu Medical Pharmaceutical Ingredients Company (China). β-CD was obtained from Shanghai Chemical Company (China). HPMC (Tianjin LE Company, China), HPMCP HP-55 (Shin-Etsu Chemical Co., Ltd, Japan), Eudragit L100/S100 (Röhm GmbH & Co. KG) were used as pH dependent coating materials. Centrifugal granulator (Type BZJ-360M, Tianmin High Technique and Development Company Beijing, China). Hartley male guinea pigs were supplied by the Animal Center of Shenyang Pharmaceutical University. Methanol was HPLC grade and other reagents were analytical grade.

Preparation of Fine Intermediate Product

The β-CD inclusion complexes of *Styrax* and *Borneolumsyntheticum* were prepared by the method of ''dropwise,''[14] with the weight ratio of β-CD to *Styrax* at 3:1 (w/w) and *Borneolumsyntheticum* at 1:1 (w/w). *Radix aristolochiae*, *Olbanum and Lignum santali albi* were crushed into coarse powder (24 mesh) respectively and mixed together. By way of water steam distillation, volatile oil was extracted from the above mixture and then made into β-CD inclusion complex in the same method at a ratio of 1:10 (ml:g)for oil and β-CD. The residue was extracted twice with 80% ethanol. Drain the liquid from the dregs and then mix the water extract liquid and the ethanol extract liquid. The dried extracts were obtained by spray drying after solvent removal under the reduced pressure. Add one fold of MCC into the dried extracts as a diluent. Finally the fine intermediate product was obtained by mixing the above three kinds

of β-CD inclusion complexes and the dried extracts before smashing them into fine powder (120 mesh).

Preparation of Pellets

The pellets were prepared in a centrifugal granulator using the powder layering technique.[15] Used as pellet cores, 500 g self-made MCC nonpareil beads (40–60 mesh) were charged into the granulator's revolving chamber. The layering powder (5 kg) contained 75% of the fine intermediate product and 25% of MCC. HPMC (5 mPa s) aqueous solution (3%) was used as the binding solution. The powder layering process was performed under the following parameters: rotor speed: 150 rpm, binder rate: 15 rpm, atomizing air pressure: 0.5 MPa, powder feeding rate: 10 rpm, slit air temperature: 20∼55°C,slit air volume: 12×20L/min. After dried at room temperature for 1 day, the pellets, ranging in diameter between 18–20 mesh, were selected by sieving for further coating.

Coating Process and the Preparation of GSSC

The pellets were divided into 3 equal weight portions and coated with the following pH-dependent materials separately: 1) 6% HPMC solution in 60% ethanol; 2) 4% HPMCP HP-55 solution in 80% ethanol (containing triethyl citrate and talc); 3) mix Eudragit L100 with Eudragit S100 at a ratio of 1:4(w/w) in a water solution (containing sodium lauryl sulphate, diethyl phthalate and talc). The coating process was carried out in the same equipment as powder layering process at the following conditions: atomizing air pressure : 0.5 MPa, rotor speed: 150 rpm, coating suspension feed rate: 15 rpm, split air flow volumn: 20×20 l/min and slit air temperature: 20∼45°C. The resultant products were dried in an hot air oven at 50° for 24 h.The coating level of three kinds of pellets was 15% each, which is calculated from the weight difference between the coated and the uncoated pellets and was based on the polymer weight gain.

The above three kinds of coated pellets were mixed at the weight ratio of 1:1:1, and filled into capsules to form a pH dependent sustained-release pellets system, i.e. Guanxin Suhe Sustained-release Capsules.

In Vitro Analytical Method

RP-HPLC was used for the quantitative in vitro dissolution analysis of cinnamic acid. The HPLC system (HITACHI) consisted of L-7110 pump, D-7000 interface and L-7200 auto sampler. The stationary phase was a Diamonsil C_{18} (200×4.6 mm, particle size:5 μm) column and the mobile phase was a mixture of methanol: water: acetic acid (28%: 72%: 0.2%,v/v/v). The flow rate was 0.8 ml/min with an injection volume of 10 μl. All samples were monitored by a L-7420 UV-VIS detector set at 272 nm. The retention time was 5.6 min for cinnamic acid.

GC was used for the quantitative analysis of borneol, the major ingredient in Borneolum syntheticum. The SHIMADZU GC-17A gas chromatographer was equipped with a flame ionization detector and a column packed with 5% SE-30 as the stationary phase. The instrument temperatures were set as follows: injector temperature at 140°C and detector temperature at 200°C. The carrier gas was nitrogen and the flow rate was 25 ml/min. The internal standard was naphthaline.

In Vitro Dissolution of the Coated Pellets

The in vitro dissolution behavior of coated pellets was assessed by measuring cinnamic acid released in the various dissolution media. Using the Ch.P 2000 Type II rotating paddle method, the tests were started with 800 ml of 0.1 M HCl, which was then changed to pH5.8 by adding 65∼75 ml of PBS (10 g of KH_2PO_4, 4 g of NaOH →100 ml) after 2 h, and to pH7.0 by adding 15∼25 ml of the same PBS after 4 h. All the tests were maintained at 37.0±0.5°C, with a paddle rotation speed of 50 rpm. 5 ml samples (replaced) were withdrawn at various predetermined time intervals. The released cinnamic acid was determined by a RP-HPLC method.

Solubility Study and Dissolution Rate Study of Borneolum Syntheticum and Borneolum Syntheticum/β-CD Inclusion Complex

The solubility of Borneolum syntheticum and Borneolum syntheticum/β-CD inclusion complex was determined by the shake-flask method. First, an excess amount of Borneolum syntheticum or Borneolum syntheticum/β-CD inclusion complex was added into the aqueous solutions. Then shake for 3 days at 37°C before being filtered through a 0.45 μm cellulose filter. The filtrate was extracted with ethyl acetate and then analyzed by a GC method.

The dissolution behavior of Borneolum syntheticum and its inclusion complex was examined in water according to the Ch.P 2000 Type II rotating paddle method. Each sample containing an equivalent amount of 20 mg borneol was introduced into 900 ml of water,

maintained at 37°C and stirred at 100 rpm. At predetermined time intervals, 3 ml samples (replaced) was withdrawn and filtered through a 0.45 μm cellulose filter. The filtrate was extracted with ethyl acetate and analyzed by a GC method.

Preparation and Analysis of Blood Samples

Preparation of Drug-Containing Human Plasma and Serum and the Determination of Cinnamic Acid

Approved by the local Ethnics Committee, the in vivo studies were performed in 12 healthy male volunteers, who are 21∼23 years old, 54∼56 kg in weight and in good physical conditions according to the findings of clinical laboratory test after giving written informed consent. The study was conducted as a double blind, randomized, crossover design. After an overnight fast of at least 10 hours, each volunteer received randomly a single dose of either the test or the control formulation along with 200 ml of water. No food was allowed until a standard meal was served 4 hours after administration. Two blood samples (6 ml) were drawn immediately before (0) and at 0.08, 0.25, 0.77, 1, 2, 3, 4, 6, 8 and 12 h after dosing. One of the samples was separated to collect plasmas and the other was separated to collect sera. After a washout period of 7 days, the study was repeated in the same manner in order to complete the crossover design. The plasmas and sera were kept frozen at −20°C until being assayed.

The human sera were used to carry out the coronary blood flow experiments in isolated guinea pig hearts. The time to pour each serum into the isolated guinea pig heart matched the time when the serum was collected from the volunteers. Based on the experimental results, effect-time curves were drawn and the related parameters were calculated.

An HPLC method was developed and validated for cinnamic acid assay in human plasma samples. The stationary phase was a Diamonsil C_{18} column (200 × 4.6 mm, particle size 5 μm). The mobile phase consisted of 30% acetonitrile and 70% 0.1 mol/L KH_2PO_4 buffer solution and was pumped at a flow rate of 1.2 ml/min. Eluent was monitored by a UV detector set at 272 nm.

Into 1 ml of plasma, 100 μl 1 mol/L NaOH and 50 μl of internal standard solution (3-(3-methylphenyloxy), 50 μg/ml in methanol) were added and the mixture was heated on a 50°C water bath for 0.5 h. Then 100 μl of 3 mol/L H_3PO_4 was added. After vigorous mixing, 3 ml extracting solution (hexane: chloroform: isopropanol, 15/10/1, v/v/v) was added into the mixture. Then the mixture was mixed and centrifuged at 3500 r/min for 10 min before the supernatant was transferred into a new glass tube and evaporated to dryness in 40°C water bath under N_2 flow. The residue was dissolved in 100 μl of mobile phase. Then 20 μl was drawn and injected into the HPLC column.

Preparation of Drug-Containing Guinea Pig Serum

Following the Regulation of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, 12 Hartley male guinea pigs weighing 300∼350 g were randomly divided into four groups: one control group and three GSSC intragastric administration groups. The GSSC groups were administered twice daily for 3 days at a dosage of 650,1950 and 3900 mg/kg, which was 5,15 and 30 times the equivalent dose of the daily clinical oral administration dose in humans. The blood was collected one hour after the last administration and centrifuged for 20 minutes at 2500 rpm. Combine the sera of the same group, mix and store at −20°. Collect and separate the blood samples from the control group to obtain the control serum.

The guinea pig sera were used to carry out the coronary blood flow experiments in isolated guinea pig hearts. Based on the experimental results, dosage-effect curve was drawn.

Determination of Coronary Blood Flow in Isolated Guinea Pig Hearts

Under light ether anaesthesia, male Hartley guinea pigs were killed by hitting the heads and the hearts were explanted rapidly. The isolated guinea pig hearts were prepared via Langendorff method and then perfused with oxygen-saturated Krobs–Ringer solution at constant temperature and pressure. (Preparation of Krobs–Ringer solution: NaCl 19 g, KCl 0.42 g, $NaHCO_3$ 0.5 g, $CaCl_2$ 0.24 g, glucose 1 g, add distilled water to 1000 ml.) After 30 min to 40 min's stabilization, 1 ml serum prepared under 2.8.1 or 2.8.2 was perfused into the isolated guinea pig hearts through the perfusing system. The coronary blood flow per minute within 5 min was determined and the maximum increase percentage of flow after administration was calculated as follows.

$$F_{max} = \frac{F_a - F_b}{F_b} \cdot 100\%$$

where F_{max}: maximum increase percentage of flow.

F_a: maximum value of coronary blood flow after administration (ml).

F_b: average value of coronary blood flow before administration (ml).

RESULTS AND DISCUSSION

Preparation of Uncoated Pellets

During the development of a dosage form containing herbal extract, one has to take into account that the components in a TCMCR have different physicochemical properties. So methods must be developed to minimize these differences. The formation of an inclusion complex of drug has the advantages in enhancing the drug's dissolution proportions, reducing the drug's volatility and changing the liquid state to the solid state etc.[16,17] Therefore, several β-CD inclusion complexes were prepared in this study to improve the properties of crude drugs.

Styrax is a mucoid oil of high viscosity and stickiness. It is difficult to prepare pellets out of such a sticky material. To solve this problem and facilitate the application of styrax, the *styrax*/β-CD inclusion complex was prepared at a weight ratio of 1:3. The amount of β-CD, which is as low as possible to decrease the dosage volume, was determined by factorial design and ANOVA analysis.

Borneolum syntheticum is characterized by volatility and low aqueous solubility. In order to increase its aqueous solubility and reduce its volatility, inclusion complex were prepared at a weight ratio of 1:1 for Borneolum syntheticum and β-CD. The ratio was determined by factorial design and ANOVA analysis. The solubility and dissolution rate in water were determined for both Borneolum syntheticum and Borneolum syntheticum/β-CD inclusion complex by measuring its major component-borneol. Compared with 504.2 μg/ml of borneol dissolved in water, the results showed that the solubility of inclusion complex was increased up to 686.5 μg/ml. It also clearly revealed an enhancement in the dissolution rate of the inclusion complex, i.e. when 100% of the borneol in the inclusion complex was dissolved in 10 min in water, only 80% of borneol was dissolved in 2 h.

Extracted from the mixture of *Olbanum*, *Radix aristolochiae* and *Lignum santali albi* using water steam distillation method, the obtained oil was of volatility and thus also made into β-CD inclusion complex at a ratio of 1:10 (ml:g) to reduce its volatility and maintain better its therapeutic effect.

After the mixture of *Olbanum, Radix aristolochiae* and *Lignum santali albi* was extracted in water and 80% ethanol in turn, the dried extracts were obtained by vacuum drying at 60° for 5 days. Upon crushing, the dried extracts were hygroscopic and of viscosity. The addition of one fold MCC as a diluent into the dried extracts powder was intended to decrease its viscosity. To find the best diluent, MCC, starch and dextrin were tried at different ratios. All of them showed a certain effect in reducing viscosity of the dried extracts. But the pellets produced showed different characters in surface morphology, friability, etc. Taking a holistic view, we chose one fold of MCC as an ideal diluent .

By mixing the above three kinds of β-CD inclusion complexes and the dried extracts, the fine intermediate product was obtained by smashing them into fine powder (120 mesh), which was of good performance in flowability and suitable for preparing pellets by powder layering technique.

BZJ-36M centrifugal granulator was an appropriate machine for powder layering process. Keeping the balance between the feeding rate of the layering powder and the binder solution is very important. The feeding rate of powder and binder was optimized in order to prevent agglomeration and powder loss. Microscopic evaluation of pellets was performed by observing optical images. According to visual observation, the surface of pellets is smooth, indicating that the balance of powder feeding and the binder addition were in good conditions in our study.

Preparation of pH-Dependent Gradient Release Pellets

In developing oral drug delivery devices that can control the amount of drug release in a defined period of time in gastrointestinal tract, one of the methods is to mix pellets coated with different pH-sensitive coating materials. By doing so, it is expected the drugs in the pellets would be released in different parts of gastrointestinal tract with the process of time, and a prolonged effect will be exhibited.[18] In this paper, the pH-dependent sustained-release system was prepared by coating pellets with HPMC, HPMCP HP-55 and Eudragit L100/S100 respectively. The above three kinds of coated pellets were designed to release drug in stomach, duodenum and jejunum or ileum, separately. Thus the result of instant onset, sustained release and high efficacy would be achieved.

pH-Dependent Dissolution Study of the Coated Pellets

Due to a lot of components with quite different physical and chemical properties in TCMCR, how to

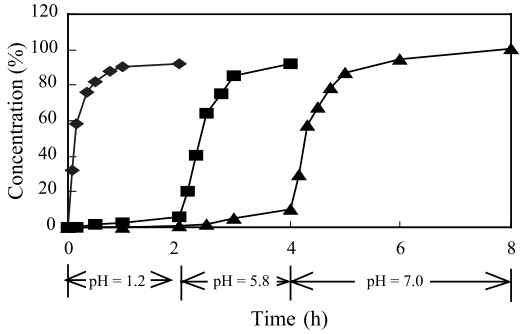

Figure 1. The dissolution profiles of cinnamic acid from differently coated pellets. Key (◆) pellets coated with HPMC; (■) pellets coated with HPMCP HP-55; (▲) pellets coated with Eudragit L100/S100.

evaluate in vitro release and PK characteristics is a very difficult problem. Since the therapeutic effects of orally administered drugs in solid forms are largely dependent on the absorption of principal components, therefore, cinnamic acid, which is the active component of *styrax* was chosen as the marker and measured as the quality index to conduct the in vitro dissolution study and the pharmacokinetic study.

The individual in vitro dissolution profiles of the three kinds of coated pellets were shown in Fig. 1. For pellets coated with HPMC, 92.3% of the cinnamic acid dissolved in 0.1 M HCl in 2 hours while for pellets coated with HPMCP HP-55, little (5.6%) dissolved in 0.1 M HCl in 2 hours and 92% dissolved in pH5.8 Phosphate Buffer Solution (PBS) in another 2 hours. The dissolution profiles of Eudragit L100/S100 coated pellets were also similar to that of HPMCP HP-55, with 0.97% dissolved in 0.1 M HCl in 2 hours, 10.5% dissolved in pH 5.8 PBS in another 2 hours and 100.4% dissolved in pH7.0 PBS in 8 hours. When the above three kinds of coated pellets were mixed at the weight ratio of 1:1:1, the dissolution profile (Fig. 2) was of a typical sustained-release character.

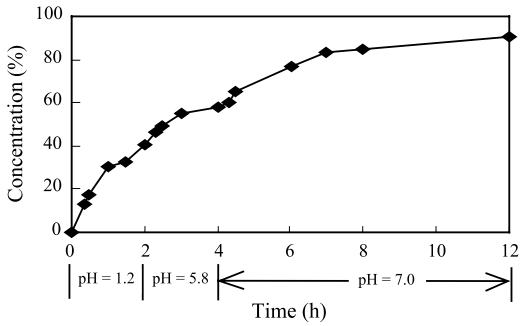

Figure 2. The release profile of cinnamic acid from Guanxin Suhe sustained-released capsule in the simulated gastrointestinal pH conditions.

Pharmacokinetic Studies of GSSC

Plasma Cinnamic Acid Concentration Method

The plasma cinnamic acid concentration-time profiles of ''Guanxin Suhe Wan'' and ''Guanxin Suhe Sustained-release Capsules'' in healthy male volunteers are shown in Fig. 3. The compartment-based data fitting of GSW complied with a two compartment model, showing a quick absorption. However for GSSC, the in vivo process of cinnamic acid is complicated, showing 2 or 3 peaks because pH-dependent coated pellets released cinnamic acid at different GI tract positions as time processed. To facilitate PK comparison between the 2 preparations, the pharmacokinetic parameters were calculated using non-compartmental model. The area under the plasma concentration versus time curve (AUC) and area under the first moment curve (AUMC) were calculated according to the trapezoidal rule. Mean residence time (MRT) was calculated according to the equation MRT=AUMC/AUC. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the plasma concentration-time profile. Relative bioavailability (F_{rel}) was calculated by: $F_{rel}=AUC_T/AUC_R$, where T: tested drug, R: reference drug

After administration, the mean plasma level of cinnamic acid released from GSW rose quickly and

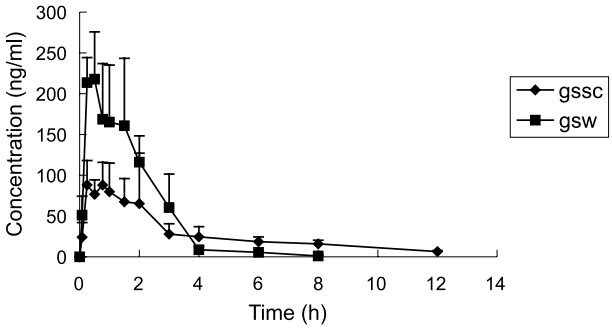

Figure 3. Mean cinnamic acid plasma concentration-time profiles following oral administration of ''Guanxin Suhe Wan'' and ''Guanxin Suhe sustained-release capsules'' in 12 healthy male volunteers.

Table 1. The main pharmacokinetic parameters of plasma cinnamic acid after a single oral administration of the two formulations in healthy male volunteers (mean ± SD).

Parameter	Guanxin Suhe Wan	Guanxin Suhe sustained release capsules
T_{max}/h	0.42 ± 0.14	1.08 ± 0.88
C_{max}/ng · mL^{-1}	252.44 ± 32.1	124.63 ± 10.97
$AUC_{0\sim\infty}$/ng · mL^{-1} · h	523.03 ± 90.64	500.12 ± 61.89
$AUMC_{0-\infty}$/ng · mL^{-1} · h^2	1113.32 ± 128.17	5412.75 ± 83.49
MRT/h	2.15 ± 0.15	11.06 ± 1.55
F_{rel}(%)	100	95.62

C_{max} and T_{max} was 252.44 ng/ml and 0.42 h respectively. There was a major fall in plasma drug concentration between 0.39 and 4 h. After 4 h, the cinnamic acid in plasma could hardly be detected. On the other hand, after the administration of the sustained-release preparation—GSSC, C_{max} and T_{max} was 124.63 ng/ml and 1.08 h respectively and the mean plasma level of cinnamic acid changed indistinctly from 0.25 h to 3 h. The concentration decrease of cinnamic acid occurred at a lower rate than that released from the GSW. As shown in Table 1, the MRT of the sustained-release preparation was much longer than that of ''Guanxin Suhe Wan,'' whereas no significant difference was observed in AUCs obtained from each praparation. In plasma drug concentration-time profile, a characteristic gradient-release of cinnamic acid can be found for GSSC.

Serum Pharmacology Method

Another method used specifically in assessing the PK behavior of TCMCR is based on the serum pharmacological method,[19] which is an in vitro method of applying drug-containing serum to isolated reaction systems. The serum is collected and separated after a period of oral intake or perfusion of TCMCR. Considering the complex chemical constituents in TCMCR and the fact that most compound preparations are absorbed in the GI tract after oral administration and then take on effects after a series of in vivo biotransformation processes, drug-containing serum mimics more closely the interior (in vivo) conditions in which drugs perform pharmacological effects.

Determination of Effect-Time Curve and the Calculation of Related Parameters

The effect-time curves (Fig. 4) were plotted with the values obtained by subtracting the average maximum increase percentage after administration of blank control human sera from the average maximum increase percentage after administration of drug-containing human sera respectively. The PK parameters (Table 2) of GSSC and GSW were calculated in the same method as described under 3.4.1.

The mean residence time (MRT) of GSW and GSSC was 2.23 h and 9.96 h, respectively, which illustrated that the retention time of GSSC was longer than that of GSW. The effect-time curve also indicated that GSSC achieved the result of sustained-release.

Determination of Dosage-Effect Curve

The intensity of GSSC-containing guinea pig serums on the coronary blood flow of isolated guinea pig hearts at different doses was of dose-dependent linear correlation (Fig. 5), with the following dosage-effect function: $Y = 31.32 + 1.358X$, $r = 0.9994$, $n = 10$

The minimum effective dosage: $X_{min} = 45.62$ mg/kg.

Comparison Between Two the Pharmacokinetic Studies

After comparing the parameters of GSSC obtained by serum pharmacological method (Table 2) with the

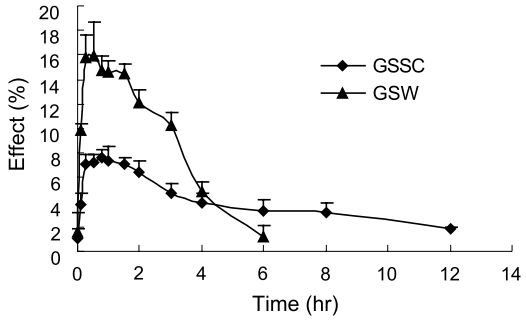

Figure 4. The effect–time curves of GSSC and GSW obtained by perfusing human drug serum into isolated guinea pig hearts.

Table 2. The statistical moments of GSSC and GSW in humans by serum pharmacological method (mean ± SD).

Parameter	Guanxin Suhe Wan	Guanxin Suhe sustained release capsules
T_{max}/h	0.56 ± 0.17	0.52 ± 0.13
E_{max}/%	17.72 ± 6.23	7.70 ± 1.75
AUC/h*%	54.86 ± 11.23	66.83 ± 10.33
MRT/h	2.23 ± 0.95	9.96 ± 2.37
F_{rel}(%)	100	121.82

plasma drug concentration method (Table 1), we found the F_{re1} obtained by serum pharmacological method (121.82%) was higher than that obtained by plasma drug concentration method(95.62%) while the MRT (9.96 h) was a little lower than that obtained by plasma drug concentration method (11.06 h).

The difference of analytical results obtained from the above two methods was hypothesized as follows: plasma drug concentration method is based on only measuring the plasma cinnamic acid concentration-time changes, while serum pharmacology study is based on the total synergistic effect of components in a compound preparation. Considering the chemical complexity of ingredients in a compound preparation, measuring the PK parameters of a single chemical moiety might not represent the actual in vivo process of other ingredients. In order to elucidate objectively the quantitative changes of a compound preparation in the physiological conditions, it is an ideal choice to conduct serum pharmacology studies on TCMCR. The curves obtained are of similar contour to those obtained by plasma drug concentration method, except a longer reaction time. This may be due to the result of joint action among chemical ingredients in a compound preparation. Thus the in vivo performance of cinnamic acid can basically reflect the pharmacokinetics of GSSC.

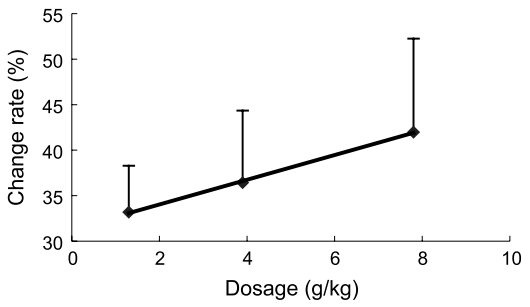

Figure 5. Dosage–change rate curve of GSSC obtained by perfusing guinea pig drug serum into isolated guinea pig hearts.

CONCLUSION

In this study, the preparation of β-CD inclusion complexes to minimize the differences in the physicochemical properties of TCMCR components has a practical meaning. The pH-dependent gradient-release pellets prepared by coating three different polymers using powder layering technique were proved to reach sustained-release both in vitro and in vivo. As different coating pellets had different sustained release times, GSSC, the modified dosage form of GSW, showed both a rapid and long effect as a whole. This system shows promising features for further application in the preparation of a variety of sustained-release TCMCR preparations.

REFERENCES

1. Roeder, E. Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie **2000**, *55*, 711–726.
2. Huang, P.L.; Huang, P.L.; Huang, P. Chem. Ind. **1992**, *20*, 290.
3. Gong, X.; Sucher, N.J. Stroke therapy in traditional Chinese medicine (TCM): prospects for drug discovery and development. Trends Pharmacol. Sci. **1999**, *20*, 191–196.
4. Griffith, V. Aust. J. Holist. Nurs. **1999**, *6*, 35.
5. Nestler, G. Traditional Chinese medicine. Med. Clin. North Am. **2002**, *86*, 63–73.
6. Leng, F.N.; Zhong, Z.J.; Yao, C. Renmin Weisheng Publishing House: Beijing, China, 1988.
7. Li, S.Z.; Wang, D.Z.; Li, X.Y. Discussion on modernization of research and development of TCM. Chin. Trade Herb. Drugs **2001**, *32*, 383–387.
8. Xu, L.Y.; Tao, J.S.; Feng, Y. Developments in pharmacy of Chinese herbal medicine in retrospect. Chin. Trade Pat. Med. **2000**, *22* (1), 6–21.
9. Zhou, H.J.; Lu, Y.R. Phytotherapy in China and its prospects. J. Chin. Pharm. Sci. **1992**, *1*, 3–12.

10. Yuan, R.; Lin, Y. Traditional Chinese medicine: an approach to scientific proof and clinical validation. Pharm. Ther. **2000**, *86*, 191–198.
11. Ch. P (2000, Part I) 544.
12. Da Ci Dian, Z.Y. Jiangsu New Medicinal College, 1975; p. 1085.
13. William, J. *Elliott Timing Treatment to the Rhythm of Disease*, 2nd Ed.; Postgraduate Medicine, 2001; Vol. 110.
14. Frömming, K.-H.; Szejtli, J. *Topics in Inclusion Science; Cyclodextrins in Pharmacy*; 1994; p. 85.
15. Muskó, Zs.; et al. Study of in vitro and in vivo dissolution of theophylline from film-coated pellets. Eur. J. Pharm. Biopharm. **2001**, *51*, 143–146.
16. Loftsson, T.; Brewster, M.E. Pharmaceutical applications of cyclodestrins 1. drug solubilization and stabilization. J. Pharm. Sci. **1996**, *85*, 1017–1025.
17. Brewster, M.E.; Loftsson, T. The use of chemically modified cyclodextrins in the development of formulations for chemical delivery systems. Pharmazie **2002**, *57* (2), 94–101.
18. Chapter 1 Drugs and the Pharmaceutical Sciences. In *Novel Drug Delivery Systems*, 2nd Ed.; Chien, Y. W., Ed.; Marcel Dekker, Inc.: New York City, 1991; Vol 50.
19. Santang, Z. *Research Progresses in the Pharmacokinetics of TCMCR J*, 3rd Ed.; ANHUI TCM COLLEGE, 2000; Vol. 19, 58–61.