

Evaluation of Tetrandrine Sustained Release Calcium Alginate Gel Beads *In Vitro* and *In Vivo*

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An approach for the preparation of tetrandrine sustained release calcium alginate gel beads was described. *In vitro* the release of tetrandrine from sustained release dosage forms went on a time of 12 hours which fitted non-Fickian diffusion with matrix erosion significantly. *In vivo* the plasma concentration of tetrandrine extended preparation given in dogs reached C_{\max} $2.67 \pm 0.69 \mu\text{g/ml}$ approximately at 5.67 ± 0.58 h after oral administration. The $\text{AUC}_{0 \rightarrow 24}$ and $\text{AUC}_{0 \rightarrow \infty}$ were $24.64 \pm 6.77 \text{ mg} \cdot \text{h/l}$ and $29.75 \pm 5.30 \text{ mg} \cdot \text{h/l}$, respectively. The elimination half-time was 9.6 ± 2.40 h. While a favorable correlativity existed between *in vitro* and *in vivo* with a correlative coefficient of 0.9798 through linear regression. An investigation on the quantitative relationship between *in vitro* release and *in vivo* absorption is a highly necessary work guided for manufacture, optimization and *in vivo* evaluation of sustained release dosage by means of *in vitro* release or dissolution tests.

Key words—tetrandrine; calcium alginate; sustained release; *in vitro*; *in vivo*

INTRODUCTION

Tetrandrine (TET) is an important bisbenzylisoquinoline alkaloid isolated from the bulbous root of *Stephania tetrandra* S. Moore of the *Menispermaceae* family known as “hanfangji”, which is one of Chinese herbal medicine traditionally used for the treatment of edema, rheumatic disorders, and inflammatory diseases. TET, its mainly active component, is qualified with obviously pharmacological action including anti-inflammatory, anti-allergic, antioxidant, anti-hepatic fibrosis as well as being effective in the treatment of arrhythmia, hypertension, silicosis, and so on.¹⁾

Besides plenty of pharmacological effects mentioned above together with some pharmacokinetics and analytical methods of TET raw material reported,²⁻⁴⁾ quite a few pharmacokinetic researches of TET oral sustained release preparation were carried on. In this study we primarily investigated the *in vitro* drug release from TET sustained release calcium alginate gel beads and its absorption *in vivo*, and consequently looked for the correlativity for both. The establishment of a relationship between *in vitro* and *in vivo* is the main purpose, which is instructional and convenient for further clinical research towards sus-

tained or controlled release preparations.

MATERIALS AND METHODS

Materials TET and theophylline standard were obtained from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). TET raw material (purity > 98%) was purchased from Xi'an Huike Bio-Tech Co. Ltd. (Xi'an, China). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, USA) and other chemicals and solvents were all of analytical-reagent grade.

Instrumentation The HPLC systems (HITACHI, Japan) were equipped with L-7110 HPLC pump and L-7420 ultraviolet detector coupled with a HW-2000 analytical workstation (Nanjing Qianpu Software Co. Ltd., China).

HPLC Conditions Liquid chromatographic analysis was performed on a Hypersil ODS C_{18} analytical column (4.6 mm × 250 mm i.d., 5 μm , Elite Analytical Instruments Co. Ltd., Dalian China) with a guard column YWG C_{18} (30 mm × 4.6 mm) at 25°C. The mobile phase was consisted of acetonitrile: pH3.6 acetate buffer solution (10: 90, v/v) at a flow rate of 1.0 ml/min. Under the conditions TET and theophylline (used as internal standard) detected at 270nm were eluted at approximately 4.78 and 8.86 min without significant interference in blank dog plas-

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ma. The HPLC method introduced had already been of validation.⁵⁾

Sample Preparation A 1.0 ml aliquot of each plasma sample was transferred to 10 ml centrifugal tube with a fixed amount of internal standard (100 μ l); then 4.0 ml dichloromethane was added to the tube and vortexed for 1 min. After centrifugation (10 min at 6000 r/min) the supernatant was transferred to a fresh tube and evaporated to dryness at 45°C in nitrogen atmosphere. The final residue was dissolved in 100 μ l mobile phase with vortex-mixing for 30 s. An aliquot of 20 μ l was subjected to the HPLC.

Preparation of TET Sustained Release Calcium Alginate Gel Beads TET sustained release calcium alginate gel beads were prepared as described previously.⁶⁾ The gel beads loading TET were prepared by dropping 2.5% sodium alginate into 0.3 mol/l CaCl_2 at the rate of 30 drops/min. After a curing period of 24 h in CaCl_2 solution the beads were filtered and washed with distilled water, then dried at 40°C for 24 h. Because of the solubility of TET sensible to pH value, TET calcium alginate gel beads were coated by fluidized bed technique to extend drug release. The coating formulation was composed of Eudragit RS 30D/Eudragit RL 30D (5:1), 20% triethyl citrate (TEC) and 35% talcum. Sustained-release could be achieved as the coating weight increasing up to 45%.

Release of TET Sustained Release Calcium Alginate Gel Beads *In Vitro* As the swelling of calcium alginate gel beads and solubility of TET was influenced by pH value, 0.1 mol/l HCl solution was selected as the release medium and replaced by pH6.8 PBS 2 h later to simulate the human gastrointestinal tract.⁷⁾ The release of TET was studied using CP 2005 XC dissolution test apparatus employing a basket method. The solvent (900 ml) was maintained at $37 \pm 0.5^\circ\text{C}$, and continuously stirred at 100 ± 1 r/min. Sample aliquots were collected at prescheduled time and replaced with an equivalent volume of fresh solvent. Drug release was analyzed and calculated by ultraviolet spectrophotometer at 264 nm as reported previously.⁵⁾

Pharmacokinetics of TET Sustained Release Calcium Alginate Gel Beads *In Vivo* Healthy dogs were purchased from The General Hospital of Shenyang Military Region weighing 20 ± 2.5 kg. After an overnight fast with free access to water, the dogs were orally given a single dose of TET sustained

release calcium alginate gel beads within six capsules (100 mg/capsule) or commercial TET tablets (40 mg/tablet) as reference formulation (RF). The dogs were further fasted for 2 h following administration. About 2 ml blood sample were collected from small saphena to heparinized tubes at predetermined intervals. The blood sample were centrifuged at 3000 r/min for 5 min at room temperature; then the separated plasma was stored at -20°C until analysis within 1 months.

RESULTS AND DISCUSSIONS

***In Vitro* Studies** Profile of the mean cumulative TET from the sustained release gel beads was illustrated in Fig. 1. Three batches of products were all able to delay drug release for 12 h. During the first two hours in 0.1 mol/l HCl drug release was less than 25% and in the end nearly all of TET released, which was supposed to be a precondition for preferable bioavailability *in vivo*. From the release profile it was observed that the percentage of released drug increased rapidly after 4 h, which was owing to the swelling property of calcium alginate susceptible to the environment of pH. During the first two hours in 0.1 mol/l HCl solution, calcium alginate gel beads did not happen to swell or erode and still keep intact matrix, as well as the outer Eudragit® film-coating. Two hours later the medium was displaced by pH6.8 PBS, in which cross-linking calcium alginate gel converted to soluble sodium alginate gel by ion-exchange.⁸⁾ As a result the gel beads appeared to swell and showed further erosion, which led to the gradual break of the Eudragit® film-coating. So the released drug increased rapidly after 4 h compared with before.

Furthermore, the information obtained in the

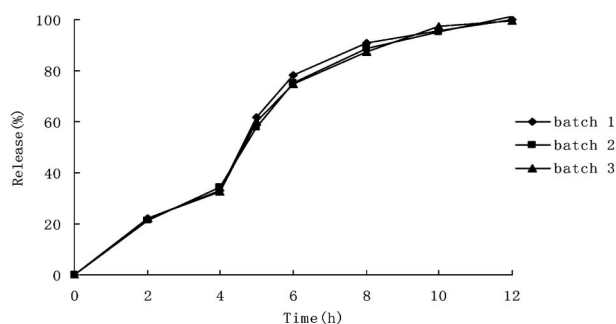


Fig. 1. TET Released Percentage from Sustained Releases Calcium Alginate Gel Beads

mathematic model of the cumulative release percent *in vitro* should be an important aid for formulators. Drug release dynamics is the key to study sustained and controlled release dosage forms, and mathematic models adopted is mainly the investigative method, such as zero-order/first-order kinetic equation, Higuchi equation, Hixson-Crowell equation and Ritger-Peppas equation.⁹⁾ Based on the fitted linear regression equation achieved from cumulative release percent *versus* time (Table 1), it is concluded that TET sustained release gel beads should meet non-Fickian diffusion, that is the co-operation of drug diffusion and hydrophilic matrix erosion. As observed in fact, the matrix of gel beads were absolute erosion eventually. Combined with the good fitted linear regression line of Hixson-Crowell equation, the matrix erosion mechanism should play an important role during the course of drug release.

In Vivo Studies C_{max} and T_{max} were the actually determined data, while AUC was calculated by trapezoidal method. Other pharmacokinetic parameters were obtained by 3P97 pharmacokinetics software and summarized in Table 2. The mean con-

centration *versus* time profiles are shown in Fig. 2. There obviously existed slow absorption and elimination for TET sustained release calcium alginate gel beads contrasted with ordinary tablets (*see* Tables 2 and 3). Following oral administration of 30 mg/kg TET, plasma levels of TET sustained release gel beads reached C_{max} $2.67 \pm 0.69 \mu\text{g/ml}$ about $5.67 \pm 0.58 \text{ h}$ after taking medicine. The $AUC_{0 \rightarrow 24}$ and $AUC_{0 \rightarrow \infty}$

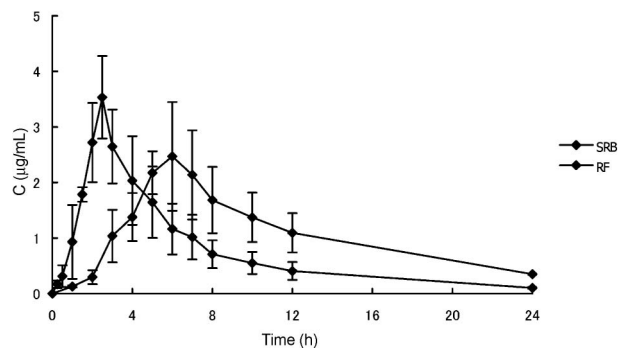


Fig. 2. Plasma Concentration Time Profile of TET in Dogs after Administration Single Dose of TET Sustained Release Gel Beads (SRB) or Tablets (RF) mean \pm S.D., $n=3$.

Table 1. Regression Results of Release Process Fitted to Different Expressions ($n=3$)

No.	Model	Equation	Corr. Coef.
1	Zero-order	$Q=11.118t$	$r=0.9474$
2	First-order	$\ln(100-Q) = -0.6588t + 6.1555$	$r=0.9312$
3	Higuchi	$Q=0.035t^{1/2}$	$r=0.9087$
4	Hixson-Crowell	$(100-Q)^{1/3} = -0.4530t + 5.3643$	$r=0.9962$
5	Ritger-Peppas	$\ln Q = 0.8403 \ln t - 1.8538$	$r=0.9831$

Table 2. Pharmacokinetic Parameters of TET in Dogs after Administered TET Sustained Release Beads ($n=3$)

No.	T_{max} (h)	C_{max} ($\mu\text{g/ml}$)	K_e (1/h)	$T_{1/2}$ (h)	$AUC_{0 \rightarrow t}$ ($\text{mg} \cdot \text{h/l}$)	$AUC_{0 \rightarrow \infty}$ ($\text{mg} \cdot \text{h/l}$)
Mean	5.67	2.67	0.07	9.60	24.64	29.75
SD	0.58	0.69	0.02	2.40	6.77	5.30

Table 3. Pharmacokinetic Parameters of TET in Dogs after Administered TET Tablets ($n=3$)

No.	T_{max} (h)	C_{max} ($\mu\text{g/ml}$)	K_e (1/h)	$T_{1/2}$ (h)	$AUC_{0 \rightarrow t}$ ($\text{mg} \cdot \text{h/l}$)	$AUC_{0 \rightarrow \infty}$ ($\text{mg} \cdot \text{h/l}$)
Mean	2.50	3.53	0.12	6.07	23.18	28.54
SD	0.00	0.74	0.02	1.12	4.79	4.76

Table 4. Cumulative Release Percentage of Sustained Release Beads *In Vitro* and Absorption Percentage *In Vivo* ($n=3$)

Time (h)	1	2	3	4	5	6	8	10	12
Release (%)	8.01	22.08	24.55	33.24	61.80	78.41	90.81	95.62	100
Absorption (%)	0.65	2.54	9.31	15.62	30.25	45.66	52.11	59.81	76.66

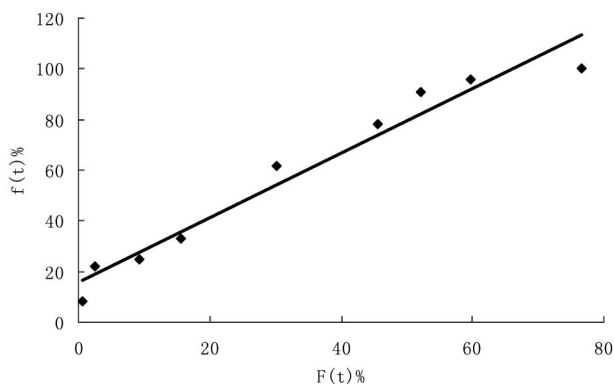


Fig. 3. *In Vivo-In Vitro* Correlation of TET Sustained Release Gel Beads

were 24.64 ± 6.77 mg·h/l, 29.75 ± 5.30 mg·h/l. The elimination half-time ($T_{1/2}$) and elimination rate constant (K_e) were 9.6 ± 2.40 h, 0.07 ± 0.02 l/h. And the relative bioavailability of TET sustained release calcium alginate gel beads was 104.24%.

Correlativity between Release *In Vitro* and Absorption *In Vivo* TET sustained release gel beads were fitted one-compartment model based on residual sum of squares, Akaike's information criterion (AIC) and r^2 obtained from 3P97 software. Consequently, absorption percents $F(t)$ were calculated by Wagner-Nelson equation.¹⁰ Correlativity *in vitro* and *in vivo* was investigated using the percent absorbed data *versus* percent released for TET sustained release gel beads (Table 4). The correlative coefficient of regress equation $f(t) = 1.276F(t) + 15.683$ was 0.9780. There existed a favorable linear regression relationship (Fig. 3).

CONCLUSIONS

The studies of release testing in medium and pharmacokinetics in dogs indicated that the desired purpose of sustained release was achieved. Plasma concentration maintained relatively constant for more than 12 hours. The release of sustained released gel beads *in vitro* was correlative with *in vivo* absorption

fraction well.

The objective of an *in vitro* and *in vivo* correlation was the application of release test to screen formulation with ideal absorption after administration partly instead of bioequivalence test.

In conclusion each drug product should be tested individually with the release test that best correlates to *in vivo* bioavailability. Since the release tests do serve as an essential component in the overall quality control of preparation manufacture and guidelines for *in vivo* absorption.

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