A Controlled Porosity Osmotic Pump System with Biphasic Release of Theophylline: Influence of Weight Gain on Its *in Vivo* Pharmacokinetics[1)]

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In our previous work, a controlled porosity osmotic pump system with biphasic release of theophylline, a system composed of a tablet-in-tablet (TNT) core and a controlled porosity coating membrane, was developed for the nocturnal therapy of asthma. Sodium phosphate and sodium chloride were selected as the osmotic agents in inner and outer layer of the TNT core respectively, and CA–PEG400–DEP (54.5%–36.4%–9.1%, w/w) was chosen as coating solution. Formulations with weight gain of 19 mg/T (mg per tablet), 9 mg/T and 6 mg/T were prepared respectively and their pharmacokinetics in beagle dogs were also studied to examine the influence of weight gain on their *in vivo* pharmacokinetics. Sustained release tablet of theophylline (SRT) was selected as reference to evaluate the *in vitro* and *in vivo* difference between conventional sustained release tablets and the developed formulation. T_{max} and mean residence time (MRT) of the developed formulations were prolonged compared to that of SRT and a satisfying bioavailability was achieved at weight gain of 6 mg/T. If applied to the chronotherapy of asthma at night, the developed formulation with a weight gain of 6 mg/T might help to reduce the inconvenience brought by too later administration of conventional dosage forms and maintain a relatively high blood drug concentration 7 h after administration.

Key words controlled porosity osmotic pump; biphasic release; theophylline; weight gain; pharmacokinetics

It is well known that asthma is a respiratory disease with apparent circadian variation in its pathogenesis.[2,3)] Human peak expiratory flow rate (PEF), an important index to evaluate ventilation functions of respiratory tract,[4)] decreases gradually from afternoon and usually reaches its bathyphase between midnight and morning[3)] and asthma attacks generally become more and more severe with the decrease of PEF. For nocturnal therapy of asthma, a drug delivery system with a "slow-fast" release pattern may be preferable. After bedtime administration, the system at first releases drug incorporated within it at a reduced speed, thus a relatively low but effective drug blood concentration may be maintained. At the time between midnight and morning, drug release from the system is promoted and drug blood concentration is then elevated to counter-act the exacerbated asthma symptoms near the PEF bathyphase. Thus, the biphasic drug delivery system may synchronize drug blood concentration with the biological requirements of asthma patients better than conventional dosage forms and provide greater patient compliance.

Theophylline is an effective compound for the treatment of asthma. Numerous articles had reported the chronotherapy of asthma with theophylline,[5,6)] but drug delivery systems with "slow-fast" theophylline release were seldom studied yet. Freichel and Lippold[7)] described an oral erosion controlled drug delivery system which could release theophylline with a late burst 18 h after dissolution. To synchronize the burst with PEF bathyphase, the system should be administrated at morning. Because of the relatively high PEF at daytime,[2)] a diurnal dose of theophylline may be not the best choice. Santus and Baker[8)] devised an OROS® system with a biphasic salbutamol releases, but, it exploited the unique solubility characteristics of salbutamol and was not applicable for theophylline. Thus, few drug delivery systems with "slow-fast" theophylline release are available for the nocturnal therapy of asthma.

In our previous work,[1)] we developed successfully an osmotic pump system with an *in vitro* "slow-fast" release of theophylline. The purpose of this work is to investigate the influence of the coating membranes thickness on the *in vivo* pharmacokinetics characteristics of the developed system in beagle dogs.

Experimental

Materials Theophylline (99.63% purity) and sustained release tablets of theophylline (SRT) were purchased from Zhong'an Pharmaceutical, China and Maitexinhua Pharmaceutical Ltd., China respectively. Authentic standard of paracetamol (National Institute for Control of Pharmaceuticals and Biological Products, China) was employed as internal standard. Sodium chloride (AR, Fangzhou chemical agent, China) and sodium phosphate (AR, Tianhua technology, China) were used as osmotic agents. Polyvidone K30 (Juyuan biological technology, China) and magnesium stearate (Mallinckrodt, U.S.A.) were applied as adhesive agent and lubricant agent respectively. Cellulose acetate (CA398-3, Eastman Chemical Company, U.S.A.) was selected as coating membrane. Polyethylene glycol 400 (BASF, Germany) was employed as channeling agent for controlling membrane porosity. Diethyl phthalate (DEP, Jinyu fine chemical, Tianjin, China) was selected as plasticizer. All other chemicals were of reagent grade. All standard solutions and dissolution media were prepared with deionized water.

Effect of pH on the Solubility of Theophylline To investigate the effect of pH on theophylline solubility, excess amount of theophylline was added to 10 ml buffers with different pH. The solutions were shaken on a shaking water bath (Luhe biochemical instruments, China) at 37±0.5 °C for 12 h. The samples were filtered through 0.45 μm microporous membranes and properly diluted with deionized water. Solubility of theophylline in the solutions was measured by UV-absorption measurement (UV2450, Shimadzu, Japan) at a wave length of 272 nm.

Tablet-in-Tablet (TNT) Core Preparation Composition of the TNT cores was listed in Table 1. PolyvidoneK30 was diluted to 20% (w/v) with 90% ethanol. Theophylline and osmotic agents were blended evenly and sieved through 80 mesh sieve (180 μm). Polyvidone K30 solution was added gradually and mixed uniformly with the mixture. The wet mess was dried at 50—60 °C for 4 h. The dried mess was powdered with mortars and passed through 80 mesh sieve. The obtained powder was lubricated with magnesium stearate. Inner layer was compressed into 250 mg tablets (TDP single station tablet machine, Tianhe, China, 8 mm round, concave punches). To prepare the TNT core, volume of the die cavity (11 mm round, concave

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punches) was adjusted equivalent to the weight of the TNT core (650 mg). Pre-weighed amount of outer layer powder (160 mg) was first placed in the die cavity as bottom layer; next, the inner layer was placed manually at the central of bottom layer; and last, the remaining volume of the die cavity was filled with 240 mg outer layer powder and compressed with a maximum compressing force of the tablet machine to obtain the TNT core. Average hardness of the TNT cores was found to be $13\pm0.5\,kg\cdot cm^{-2}$ (Tablet hardness tester, Huangpu analytical instrument, Shanghai, China). Drug content of the TNT cores was within the limits of 95—105%.

Coating Composition of the coating membrane was CA–PEG600–DEP (54.5%–36.4%–9.1%). Total concentration of ingredients in coating solution was 3.5% (w/v) and acetone was selected as solvent. Coating was carried out by spray pan coating machine with hot air blower (BY300A pan-coater, Shanghai, China). Rotation speed of the pan was set at $50\,r\cdot min^{-1}$, spray rate was fixed at $1\,ml\cdot min^{-1}$. Average weight gain after coating was controlled at different amounts to study the influence of coating thickness on theophylline release.

***In Vitro* Drug Release** *In vitro* release of theophylline from the developed formulations and SRT were studied using paddle type apparatus with 900 ml dissolution medium at a temperature of 37 ± 0.5 °C, and the agitation intensity was$100\,r\cdot min^{-1}$. The study was carried out in simulated gastric fluid (pH 1.2) for 2 h. Then the dissolution media was replaced by phosphate buffer pH 7.4 and the experiment was continued for 10 h. All experiments were repeated three times. Samples of 5 ml were withdrawn at specified time points (0 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h) and replaced with corresponding dissolution medium. Obtained samples were properly diluted and analyzed by UV-absorption measurement at the wave length of 272 nm.

HPLC Assay Method of Theophylline in Dog Plasma The HPLC system was consisted by a variable UV detector (G1314A, Angilent, U.S.) and a double pump model (G1311, Angilent, U.S.). An integrator model (Chemstation, Angilent, U.S.) was used for data acquisition and processing. The mobile phase consisted of methanol/water (20/80, v/v) was filtered (0.45 μm) prior to use, then pumped through the system at a flow rate of $1.0\,ml\cdot min^{-1}$. Twenty microliters of sample solution was injected on the column. The chromatograph run required 12 min for completion. The ultraviolet (UV) spectrum of theophylline in mobile phase was obtained with a UV-2450 spectrophotometer (Shimadzu, Japan). UV absorption of theophylline reached its maximum in the mobile phase at the wave length of 272 nm. Separation was achieved at 30 °C with a Diamonsil ODS C18 column (4.6×150 mm with a 5 μM pore size, Dikma, U.S.). A pre-column treatment of plasma samples was employed. Retention time of theophylline and internal standards were 9.1 ± 0.2 min and 4.9 ± 0.2 min respectively. The calibration curve was rectilinear in the concentration range of 0.2—20 $\mu g\cdot ml^{-1}$ ($r=0.999$). The inter-day and intro-day accuracy and precision were within an RSD 6.5%. The extraction efficacy of theophylline in plasma samples was 77.0%, 82.5% and 83.1% at low, medium and high concentration respectively. The samples were stable after stored at room temperature for 24 h or stored at below −20 °C for 15 d.

Plasma Sample Preparation For determination of theophylline, 0.5 ml of plasma was taken into a 5 ml centrifuge tube and to this 10 μl internal standard (Paracetamol, $150\,\mu g\cdot ml^{-1}$) was added and vortexed for 1 min. Three milliliters diethyl ether was added to the sample and vortexed for 5 min, then centrifuged at $8000\,r\cdot min^{-1}$ for 15 min. Two milliliters organic layer was separated and evaporated under reduced pressure at 25—28 °C. The residue was dissolved with 100 μl methanol and 20 μl of samples was injected into HPLC for analysis.

Pharmacokinetics Study To investigate the correlation between the weight gain of the developed formulations and their *in vivo* pharmacokinetics, three male beagle dogs weighing 14—16 kg were used. Dogs were fasted for 12 h from food, but not water, before each phase. On three different occasions, each dog was administered the developed formulations with a weight gain of 19 mg/T, 9 mg/T and 6 mg/T at a dose of 100 mg, and an oral gavage of 300 ml water immediately followed the drug administration. Four milliliter blood samples were taken from the jugular vein of each dog before dosing and at 2, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 27, 30 h after dosing into heparinized centrifuge tubes. Five hours after drug administration the dogs were allowed free to food and water. Plasma was separated by centrifuging the blood samples at $4000\,r\cdot min^{-1}$ for 10 min at room temperature. Obtained plasma was stored at −20 °C and brought to room temperature before determination. To compare the pharmacokinetics of the developed formulations with that of the SRT, SRT were administered orally to the same fasted dogs at a dose of 100 mg. Four milliliter blood samples were collected before dosing and at 1, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 10, 12, 24, 27, 30 h after dosing into heparinized centrifuge tubes. Plasma was separated and stored according to the abovementioned procedure. Washout period between each phase was 1 week for each dog.

***In Vivo* Data Analysis** Drug And Statistics 2.0 (Clinical evaluation and research center, Shanghai University of T.C.M, China) was used to evaluate the pharmacokinetic parameters of the developed formulations and SRT. Relative bioavailability (*Fr*) of the developed formulations was calculated according to the formula:

$$Fr=\frac{AUC_{\text{developed}}}{AUC_{\text{SRT}}} \tag{1}$$

***In Vitro* and *in Vivo* Correlation of the Developed Formulations** Wanger–Nelson equation was applied to evaluate the correlation between *in vitro* drug release and *in vivo* drug absorption.[9] Percent of *in vivo* drug absorption at time point *t* was calculated according to the following formula:

$$F=\frac{C_t+k\int_0^{\tau}C_t}{k\int_0^{\infty}C_t}\times100\% \tag{2}$$

where *k* was the elimination rate constant, C_t was the *in vivo* drug concentration at time *t*.

Table 1. Composition (%, w/w) of Tablet-in-Tablet Core

Materials	Composition (%, w/w)	
	Inner layer	Outer layer
Theophylline	20.0	12.5
Sodium chloride	—	81.5
Sodium phosphate	74.0	—
Polyvidone K30	5.0	5.0
Magnesium stearate	1.0	1.0

Results and Discussion

Effect of pH on Drug Solubility Theophylline solubility at different pH was determined and the results were showed in Table 2, it can be concluded that the solubility of theophylline does not change before pH 10.8 is reached, after which the solubility increases rapidly.

Formulation Development The purpose of this work is to develop a controlled porosity osmotic pump system for the biphasic delivery of theophylline. The developed system is composed of a tablet-in-tablet (TNT) core and a rate controlling coating membrane. Inner layer and outer layer of the TNT core are consisted of theophylline and osmotic agents (sodium phosphate and sodium chloride, respectively), and the coating membrane is composed of cellulose acetate as semipermeable membrane forming polymer, polyethylene glycol as leachable pore-forming material and diethyl phtha-

Table 2. Theophylline Solubility at Different pH ($n=3$)

pH	4.5	6.0	7.0	7.9	9.0	10.0	10.4	10.8	11.0	11.4	11.8
Solubility ($mg\cdot ml^{-1}$)	7.4±0.4	6.9±0.5	6.4±0.4	6.9±0.5	6.3±0.7	7.0±0.7	7.2±0.5	8.8±0.7	10.3±0.9	11.4±0.7	14.9±1.2

late as plasticizer. During dissolution, pores are formed in the membrane gradually following the dissolution of polyethylene glycol and water is imbibed by the TNT core from dissolution medium across the membrane. At the first stage of the biphasic release, sodium chloride in outer layer of the TNT core is dissolved and a relatively high microenviromental osmotic pressure is formed, theophylline is pumped out by the osmotic pressure. At the second stage of the biphasic release, most sodium chloride in the TNT core is released and sodium phosphate in inner layer of the TNT core becomes the main osmotic agent in the formulation. During this period, microenvironmental osmotic pressure is declined greatly due to the much lower osmotic pressure of sodium phosphate solution,[10] and microenvironmental pH is elevated due to the alkalinity of sodium phosphate. Despite the decrease of microenvironmental osmotic pressure, theophylline release from the developed formulation increased due to the promoted theophylline solubility in the alkaline microenvironmental solution.

Effect of Weight Gain on *in Vitro* Theophylline Release To investigate the effect of weight gain on theophylline release, the TNT cores were coated to get formulations with different weight gain, and the release profiles of the formulations and SRT were shown in Fig. 1. As Fig. 1 showed, all the developed formulations showed a biphasic (slow-fast) release pattern. As weight gain of the coating membrane increased from 6 mg/T to 19 mg/T, percent of theophylline release during the first 6 h and the entire process of dissolution decreased from 38% and 90% to 25% and 70%. Compared with SRT, the developed formulations showed a reversed release pattern. During the first 6 h of dissolution, release rate of the developed formulations was obviously slower than that of SRT, and during the last 6 h of dissolution, release rate of the developed formulations was apparently faster than that of SRT. This release pattern might help to maintain a relatively low blood concentration before T_{max} and prolong the time needed to reach C_{max} *in vivo*.

Effect of Weight Gain on *in Vivo* Theophylline Release In this study, the developed formulations with three different weight gain (19 mg/T, 9 mg/T, 6 mg/T) and SRT were given to three beagle dogs by oral administration at a dose of 100 mg. Plasma concentration–time curves of theophylline were shown in Fig. 2. Pharmacokinetics parameters of the developed formulations and SRT were showed in Table 3. As weight gain of the developed formulations decreased from 19 to 6 mg/T, C_{max} of the formulations increased from 1.58 to 2.88 $mg \cdot l^{-1}$ and T_{max} of the formulations changed little. Accrescence of the C_{max} with the reduction of weight gain might result from the accelerated drug release from developed formulations with a thinner coating membrane. Set SRT as reference, relative bioavailability of the developed formulations with a weight gain of 19 mg/T, 9 mg/T and 6 mg/T were 50%, 48% and 98% respectively. Compared to SRT, all developed formulations displayed higher values of T_{max} and mean residence time (*MRT*). The prolonged T_{max} or *MRT* might help to improve the inconvenience brought by too late administration of conventional dosage forms for chronotherapy of asthma at night.

Strength of mechanical destructive forces in the gastrointestinal tract of humans and dogs were reported to be 1.9 N (approximately 190 g) and 3.2 N (approximately 320 g) respectively.[11,12] To maintain the integrity of osmotic pump tablets, coating membrane of the tablets should be firm enough to resist the mechanical destructive forces. Burst strength of the exhausted coating membranes was estimated by a tablet hardness tester (Huangpu analytical instrument, Shanghai, China). After dissolution, the remained tablets were put on the hardness tester, and burst strength of the exhausted coating membrane was estimated by the pressure needed to break the coating membrane. At the weight gain of 19 mg/T, 9 mg/T and 6 mg/T, pressure needed to rupture the exhausted coating membranes was estimated to be 0.30 $kg \cdot cm^{-2}$, 0.10 $kg \cdot cm^{-2}$ and 0.05 $kg \cdot cm^{-2}$ respectively. Because surface area of the exhausted coating membranes was about 0.95 cm^2, burst strength of the exhausted coating membranes was about 300 g, 100 g and 50 g. The strength of

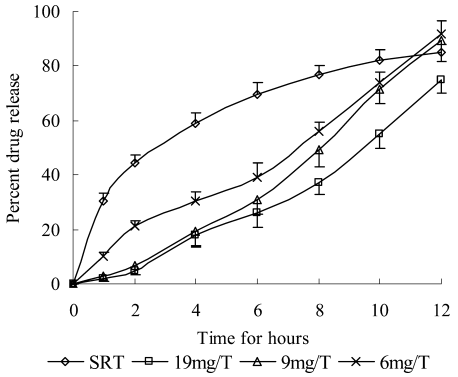

Fig. 1. Release Profiles of Developed Formulations with Different Weight Gain and Sustained Release Tablet of Theophylline

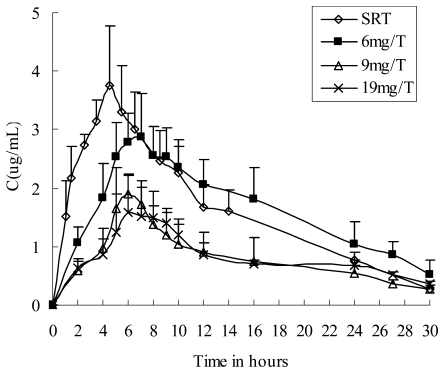

Fig. 2. Plasma Concentration–Time Curves of Theophylline after Oral Administration of Developed Formulations with Different Weight Gain and SRT

Table 3. Pharmacokinetics Parameters of Developed Formulations and Sustained Release Tablet of Theophylline (SRT)

Parameters	19 mg/T	9 mg/T	6 mg/T	SRT
$AUC_{(0-t)}$ ($mg \cdot l^{-1} \cdot h$)	23.92	23.35	47.32	48.44
$AUC_{(0-\infty)}$ ($mg \cdot l^{-1} \cdot h$)	31.85	27.84	57.67	54.74
K_a (h^{-1})	0.38	0.45	0.32	0.50
C_{max} ($mg \cdot l^{-1}$)	1.58	1.90	2.88	3.75
T_{max} (h)	6.0	6.0	7.0	4.5
$t_{1/2}$ (h)	10.52	9.37	10.22	6.90
$MRT_{(0-t)}$ (h)	13.44	12.54	13.15	10.56
$MRT_{(0-\infty)}$ (h)	22.96	17.06	16.77	12.22

Table 4. Accumulative *in Vitro* Drug Release and Accumulative *in Vivo* Drug Absorption of Developed Formulations with Different Weight Gain

Weight gain	Time (h)	2	4	6	8	10	12
19 mg/T	*In vitro* %	4.62	18.07	25.99	37.40	55.16	75.10
	F %	7.19	13.78	27.40	36.47	42.22	46.27
			$Y=0.5788X+8.0203$, $r=0.9453$				
9 mg/T	*In vitro* %	6.78	21.56	34.34	49.22	71.38	89.50
	F %	6.70	15.49	33.17	41.00	46.60	52.63
			$Y=0.5566X+7.2923$, $r=0.9563$				
6 mg/T	*In vitro* %	23.42	33.70	43.29	58.96	77.91	91.96
	F %	10.54	21.96	37.67	45.38	51.87	57.98
			$Y=0.6576X+1.4856$, $r=0.9551$				

the coating membrane with a weight gain of 9 mg/T and 6 mg/T was lower than the reported destructive forces in the dogs' gastrointestinal tract. In our research, the relative bioavailability of the formulations with a weight gain of 19 mg/T and 9 mg/T had no significant difference, which suggested that the coating membranes of the formulations remained intact in those dogs' gastrointestinal tract. The increase of the relative bioavailability of the formulations with a weight gain of 6 mg/T might due to the rupture of their coating membranes under mechanical destructive forces in gastrointestinal tract.

***In Vitro* and *in Vivo* Correlation of the Developed Formulations** Correlation between accumulative *in vitro* drug release and accumulative *in vivo* drug absorption of the developed formulations with a weight gain of 19 mg/T, 9 mg/T and 6 mg/T were showed in Table 4. The results showed that *in vivo* drug absorption of the three samples were slower but closely interrelated with their *in vitro* release. Since theophylline is absorbed rapidly after oral administration, we hypothesized that the slow *in vivo* absorption was caused by the slow *in vivo* release from the samples, and the traditional dissolution test we used in the research may be inadequate to predict the *in vivo* performance of the developed formulations well. Although the dissolution test we used is one of the standard methods accepted by the Chinese Pharmacopeia, the chemical components and physical characteristics of its dissolution medium is quite different from those of gastrointestinal fluid. The peptide, enzyme, food residue and other organic ingredients in gastrointestinal fluid may make the viscosity and osmotic pressure of it higher than those of the dissolution medium, and thus retard the water absorption and drug release of the developed formulations in gastrointestinal tact. The unsatisfying correlation between *in vitro* and *in vivo* tests of the developed formulations might be caused by the difference between common used dissolution medium and gastrointestinal fluid. To promote the correlation of the two tests, some additional regents might be added to the *in vitro* dissolution medium to make it has similar viscosity, osmotic pressure and ionic strength with gastrointestinal fluid.

Conclusion

A chronopharmaceutical theophylline delivery system for the nocturnal therapy of asthma was obtained. The developed system was composed of a tablet-in-tablet (TNT) core and a controlled porosity coating membrane. *In vitro* release of the developed system followed a biphasic "slow-fast" pattern. C_{max} of the developed formulations increased with the decreased of weight gain. Compared to SRT, T_{max} and *MRT* of the developed formulations were prolonged and drug blood concentration of the developed formulations 7 h after administration was elevated. The prolonged T_{max} or *MRT* and elevated drug–blood concentration 7 h after administration might help to postpone administration time of conventional formulations for nocturnal chronotherapy of asthma and mitigate the exaggerated symptoms of asthma better at early morning.

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