

PHARMACOKINETIC EVALUATION OF A CHRONOTHERAPEUTIC SYSTEM LOADED WITH AN ANTIASTHMATIC DRUG AS AN ORAL DRUG DELIVERY SYSTEM

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Abstract

Recent studies proved that the symptoms of bronchial asthma follow the circadian rhythms and become worse in the early morning. In such cases, conventional sustained-release treatment regimens are inappropriate for the delivery of antiasthmatic drugs as they cannot be administrated just prior to the worsening of the symptoms. The current work aimed to explore the pharmacokinetics of a model antiasthmatic drug following oral administration of a chronotherapeutic system in six healthy volunteers. A sensitive and selective chromatographic method was used to determine drug concentration in human plasma. A standard calibration curve of the drug over the concentration range of 0.15 - 80 µg/ml was prepared in human plasma. Anhydrous caffeine was used as an internal standard. The protocol of the studies was approved by the Research Ethics Committee for clinical studies at Faculty of Pharmacy, Cairo University, Egypt. Following oral administration of the treatments, the pharmacokinetic parameters of the drug including C_{max} (µg/ml), T_{max} (h), $MRT_{(0-\infty)}$ (h), AUC_{0-24} (µg h/ml) and $AUC_{0-\infty}$ (µg h/ml) were derived from the individual plasma concentration time curves. The data were statistically analyzed using two-way ANOVA at $P < 0.05$. The results confirmed that the drug reached C_{max} rapidly within two hours following a definite lag time; confirming the potential of the developed chronotherapeutic system in promoting the drug absorption at its site of action at the required time.

Keywords: antiasthmatic drug, circadian rhythm, pharmacokinetics, pulsatile drug delivery system.

Introduction

After several experiments in animals and in humans, scientists have clearly demonstrated that people vary considerably in their physiological and biochemical conditions due to the circadian rhythm. These predictable temporal cycles not only affect different physiological functions, like blood pressure or heart rate, but also can influence the pharmacokinetic phases of drugs (De Giorgi et al 2013, Baraldo 2008). In view of the aforementioned, the traditional delivery systems that promote constant delivery of the drug into the body seem unnecessary and/or undesirable. Instead, the drug release profile should be tailored to mimic a living system's pulsatile hormone

secretion, so that the drug efficacy could be improved and the possible toxicity of a specific drug regimen might be reduced (Ashwini et al 2012

Asim et al 2010 and Gandhi et al 2011). The latter approach can be addressed by chronopharmaceutical drug delivery systems which exhibit pulsatile drug release profiles that coincide with the circadian rhythms⁶ (Youan 2004).

Oral pulsatile drug delivery systems are designed to elicit programmable lag periods preceding (i) prompt, (ii) repeated or (iii) sustained release of drugs. These systems show inherent suitability for accomplishing chronotherapeutic goals, which have recently been interconnected with many chronic diseases with typical night or early morning recurrence of symptoms like bronchial asthma, cardiovascular disease or rheumatoid arthritis. In fact, the un-interrupted normal sleep patterns would be expected to ensure higher compliance (Maroni et al 2010, Ali et al 2010 and Ingersoll et al 2014).

A variety of design strategies have been attempted for pulsatile release purposes; including; solid lipid microparticles (Albertini et al 2014) , pellets (Zhang et al 2014) , osmotically-controlled capsules (Ranjan et al 2014), membrane rupture systems (Lin et al 2008), time-controlled explosion systems (Ueda et al 1994) , tabs-in-cap systems (Gangwar et al 2015), mini-tablets (Biswas et al 2014), press coated tablets (Shah et al 2015), Chronotropic systems (Gazzaniga et al 1994) and core-in-cup dry coated tablets (Efentakis et al 2011, Efentakis et al 2006).

Most of these systems allowed the release of the loaded-drug over a prolonged period of time following a definite lag period.

In the current work, the *in vivo* pharmacokinetic parameters of a model antiasthmatic drug following oral administration of a chronotherapeutic system were estimated in healthy volunteers to explore the ability of this system to allow a prompt drug release following a definite lag period.

2. Materials and Methods

2.1. Materials

Theophylline (TE) was granted by FIS Fabbrica Italiana Sintetici, (Alto de Montevecchio, Italy). Ethocel[®] Standard 100 Premium FP (ethyl cellulose) was donated by Dow Chemical Company (Midland, MI, USA). Methocel[®] E5 (HPMC 2910) and Methocel[®] K100M Premium (HPMC 2208) were grant samples from Colorcon Ltd. (Orpington, UK). Magnesium stearate was purchased from Herwe Chemisch-technische Erzeugnisse GmbH (Sinsheim-Dühren, Germany).

Acetonitrile (HPLC grade), methanol (HPLC grade), tetrahydrofuran (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, USA). Caffeine hydrochloride (CA; an internal standard) was provided by Pharco Pharmaceuticals (Alexandria, Egypt).

2.2. Establishment of a standard calibration curve of TE in human plasma

A stock solution of TE in methanol (20 µg/ml) was prepared. Another stock solution of CA (internal standard) in a 1:1 mixture of acetonitrile - methanol (5 µg/ml) was prepared. For the preparation of the calibration curve, blank plasma samples were spiked with TE to contain 0.15 - 80 µg/ml. Aliquots of spiked plasma samples (100 µl) were mixed with caffeine hydrochloride stock solution (200 µl) and the mixtures were vortexed for 30 sec and centrifuged at 8000 x g for 10 min. Portions of the supernatants

were transferred to HPLC tubes and then injected onto the Ultra performance liquid chromatography column. A standard curve was constructed by plotting the peak-area ratios of TE / CA against TE concentrations in human plasma. All assays were performed in triplicate. The lower limit of quantification was 0.15 µg/ml. The procedural constant (K) was calculated from the slope of each calibration curve.

2.3 In vivo absorption studies in healthy volunteers

2.3.1. Study design

The *in vivo* studies followed a two-treatment, two-period, randomized, crossover design to assess the pharmacokinetics of TE following oral administration of sustained release marketed tablets (Treatment A, the reference product) and the chronotherapeutic system (Treatment B) at single 100 mg doses of TE. The protocol of the studies was approved by the Research Ethics Committee for clinical studies at Faculty of Pharmacy, Cairo University, Egypt. The studies were conducted in full compliance with the declarations of Helsinki and Tokyo for human volunteers.

2.3.2. Subjects

The studies involved six healthy, non-smoking, male volunteers with no history of alcohol- or drug-abuse problems. The subjects had appropriate measures; varied in weight from 64 to 82 kg (73.5 kg), in age from 22 and 35 years (28.5 year), and in height from 166 to 188 cm (177.5 cm). A general practitioner reviewed the medical history of each volunteer and conducted a general physical examination to confirm the appropriate participation in the studies. Furthermore, the necessary hematological laboratory evaluations (total cholesterol, plasma glucose, blood urea, serum creatinine, SGOT and SGPT, hemoglobin concentration, RBC, Total Leukocyte and Platelet counts) were screened. All volunteers were instructed not to use any medicines, vitamin or herbal supplement from one week before and until the end of the studies. Following the full explanation of the nature and the purpose of the study, an informed written consent was obtained from each volunteer.

2.3.3. Administration of treatments and processing of samples

The subjects were randomly assigned to one of two groups of equal number. On phase I of the study, half the number of volunteers received one-third of oral sustained release marketed tablets 300 mg (Treatment A) while the other half of volunteers received the chronotherapeutic system (Treatment B) composed of TE (10% w/w), Ludipress® (30% w/w) in core and outer coat consisting of Methocel® (30% w/w) and ethylcellulose (30% w/w). According to FDA regulations, both treatments were ingested with 200 ml of water after 12-h overnight fasting. It should be noted that, no water was allowed one hour prior to drug administration. Yet, water was re-permitted two hours post dosing. Food and other drinks were permitted for 4 h post dosing.

Following a washout period of one week, the reverse of randomization took place on phase II. In both phases, venous blood samples (5 ml) derived from the forearm cubital veins were collected into heparinized tubes, prior to and up to 24 h post administration of treatments. The plasma samples were derived by centrifugation at 4000 ×g for 10 min and then pipetted into well-defined glass tubes (labeled with the run date, the subject code, the study period, the treatment type; reference or test) and finally frozen at -20°C until analysis.

The thawed plasma samples (100 µl) were spiked with caffeine hydrochloride (CA) internal standard solution in a 1:1 mixture of acetonitrile - methanol (200 µL; 5 µg/ml) and treated as previously reported to precipitate the plasma proteins.

2.3.4. Determination of TE concentration in human plasma by Ultra performance liquid chromatography

A sensitive and specific Ultra performance liquid chromatographic method was adopted for the quantitative determination of TE in human plasma. The mobile phase consisted of a mixture of tetrahydrofuran (30%, v/v): acetonitrile: water (10: 10: 80). It eluted through Epitomize™ Chiral CSP1 column (3.0 x 50 mm) packed with 1.7 µm particles (Orochem Technologies, Inc., IL, USA) at a flow rate of 0.5 ml/min. The peak areas of TE and CA were recorded.

2.3.5 . Pharmacokinetic and statistical analyses of data

The pharmacokinetic parameters for THE following oral administration of both treatments were determined by means of WinNonlin® software adopting the non-compartmental module. The maximum drug concentration (C_{max} , µg/ml), the time to reach C_{max} (T_{max} , h), the mean residence time from zero to infinity ($MRT_{0-\infty}$, h), the area under the plasma concentration–time curve up to 24 hour (AUC_{0-24} , µg h/ml) and upto infinity $AUC_{0-\infty}$ (µg h/ml) were determined.

The primary pharmacokinetic parameters including C_{max} , $AUC_{(0-24)}$ and $AUC_{(0-\infty)}$ were statistically analyzed, at $P < 0.05$, using two-way ANOVA.

3. Results and discussion

3.1. Linearity of the standard curve of theophylline HCl

Figure 1 shows the chromatogram of TE and CA internal standard solution in a 1:1 mixture of acetonitrile - methanol (200 µL; 5 µg/ml) in plasma. The peaks of both components were sharp and symmetrical with good base line resolution. The retention time of TE was 0.74 min while that of CA was 1.04 min. The correlation between TE / CA peak area ratio and TE concentration in plasma is shown in Figure 2. The standard curve showed good linearity over a concentration range of 0.15-80 µg/ml. The lower limit of quantification (LLOQ) that represents a signal: noise ratio of 10:1 was found to be 0.15 µg/ml. The linear regression equation was found to be:

$$y = 0.0967x + 0.0367$$

Where y is [TE / CA peak area ratio and x is THE concentration in µg/ml. K was found to be 10.341

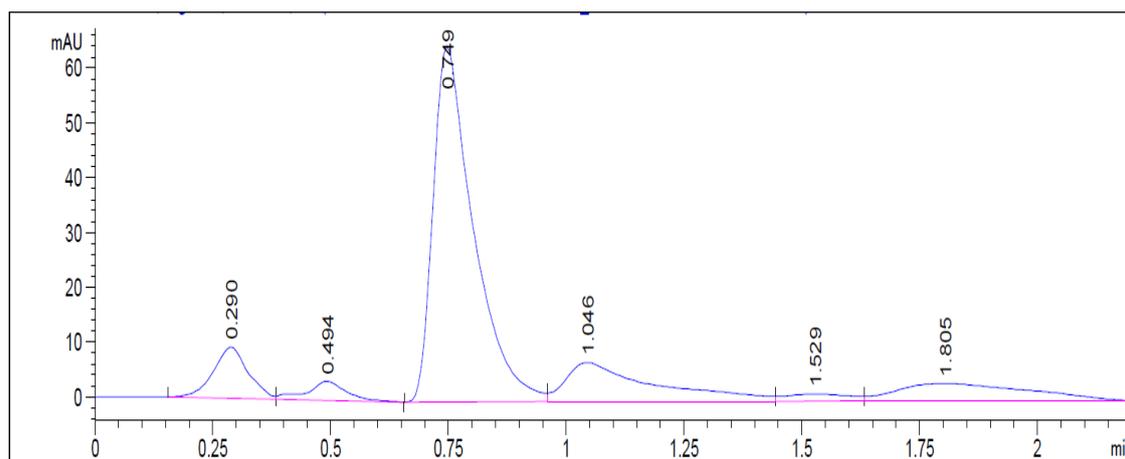


Fig. 1 A representative chromatogram of TE and CF in human plasma.

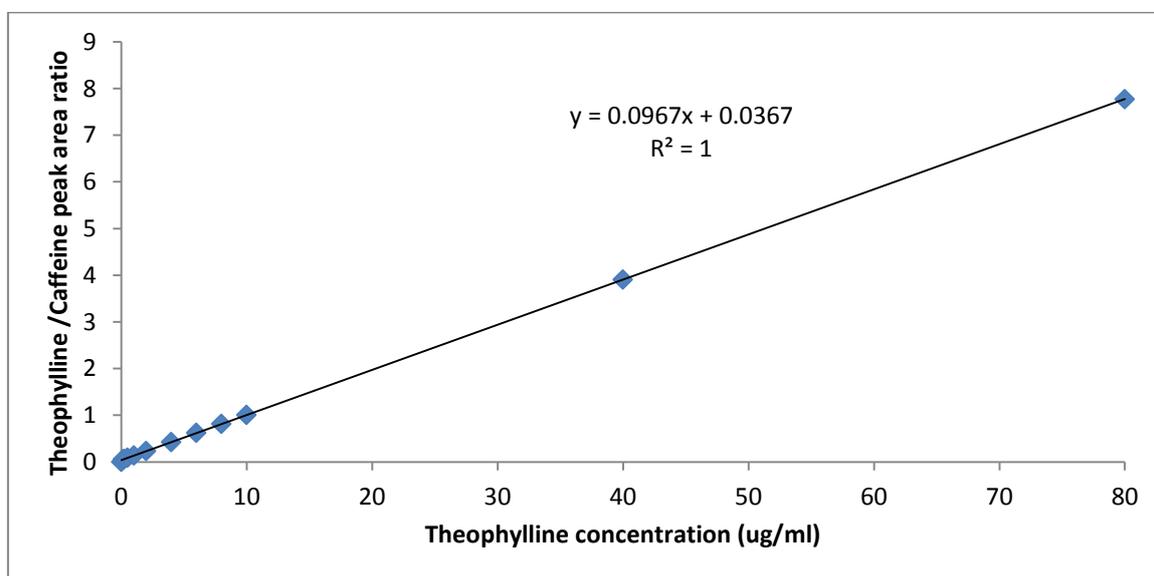


Fig. 2 The correlation between TE / CF peak area ratio and TE concentration in plasma.

3.2. *In vivo* absorption studies in healthy volunteers

The plasma concentrations of THE following oral administration of oral sustained release marketed tablets (treatment A) and TE-loaded chronotherapeutic system (treatment B) in healthy volunteers at 100 mg doses are displayed Tables (1 – 2) and Fig. 3. The pharmacokinetic parameters [C_{max} , T_{max} , $\text{MRT}_{0-\infty}$, AUC_{0-24} and $\text{AUC}_{0-\infty}$] were derived by the non-compartmental fitting of data and are summarized in Table (3).

Table 1 TE plasma concentrations ($\mu\text{g/ml}$) following oral administration of Treatment A to six healthy volunteers

Time (h)	Volunteer number						Mean \pm S.D.
	1	2	3	4	5	6	
0	1.31	1.95	0.10	0.80	1.31	1.84	1.04 \pm 0.88
0.5	3.68	2.38	0.79	1.71	3.68	3.01	2.14 \pm 1.10
1	4.43	3.70	1.26	1.48	4.43	3.70	2.72 \pm 1.88
2	8.17	7.67	4.79	9.44	8.17	1.30	7.51 \pm 3.83
4	6.82	7.82	7.06	6.00	6.82	BQL	6.92 \pm 2.96
5	5.64	7.17	6.53	4.79	5.64	BQL	6.07 \pm 2.66
6	5.03	5.75	5.07	4.76	5.03	BQL	5.15 \pm 2.06
8	2.92	4.38	4.12	2.53	2.92	1.73	3.49 \pm 1.58
9	1.32	2.38	3.49	0.58	1.32	1.60	1.94 \pm 1.14
12	1.41	1.40	0.83	0.36	1.41	0.29	1.00 \pm 1.5
24	0.72	1.08	0.01	0.52	BQL	0.43	0.52 \pm 0.42

*BQL= below quantification limit

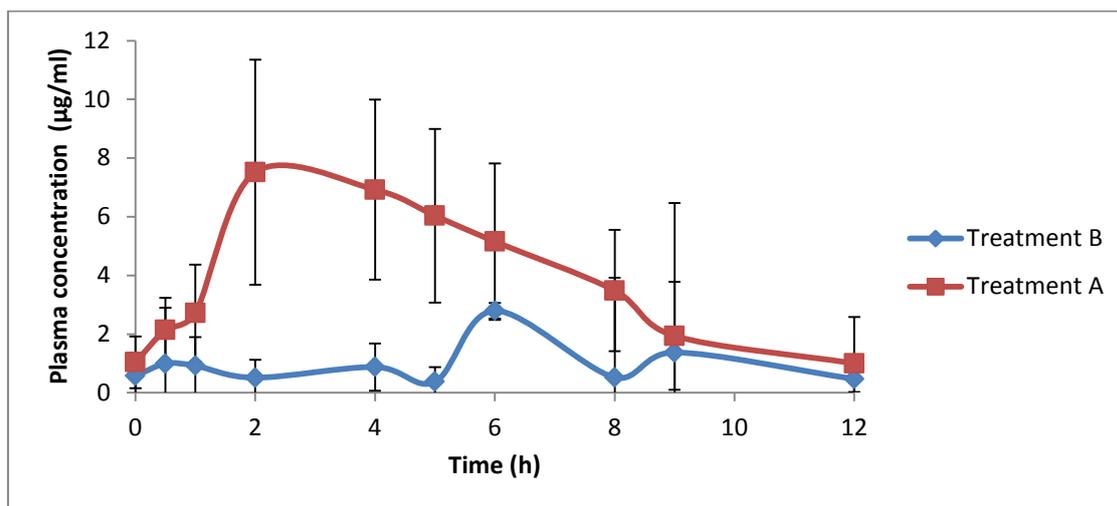
Table 2 TE plasma concentrations ($\mu\text{g/ml}$) following oral administration of Treatment B to six healthy volunteers

Time (h)	Volunteer number						Mean \pm S.D.
	1	2	3	4	5	6	
0	0.17	1.83	0.36	0.13	0.37	BQL	0.57 \pm 0.78
0.5	0.08	4.07	0.16	0.19	0.47	BQL	0.99 \pm 1.90
1	0.11	2.51	0.31	0.62	1.07	BQL	0.92 \pm 0.97
2	0.21	1.44	0.08	0.23	0.61	BQL	0.52 \pm 0.61
4	1.12	1.11	BQL	0.13	1.15	BQL	1.33 \pm 0.92
5	0.98	0.04	0.19	0.06	0.61	BQL	0.88 \pm 0.5
6	BQL	7.60	0.40	0.39	2.80	BQL	2.80 \pm 3.40
8	0.13	0.61	1.07	0.80	0.02	BQL	0.52 \pm 0.44
9	4.50	0.38	0.42	1.24	0.33	BQL	1.38 \pm 0.43
12	0.10	0.45	0.10	BQL	1.24	BQL	0.47 \pm 0.54
24	0.35	0.18	0.00	0.19	0.62	BQL	0.27 \pm 0.26

*BQL= below quantification limit

Table 3 The pharmacokinetic parameters for TE following oral administration of both treatments (mean \pm SD, n = 6).

Treatment	C _{max} (ug/ml)	T _{max} * (h)	MRT (0-∞) (h)	AUC (0-24) (ug.h/ml)	AUC (0-∞) (ug.h /ml)
A	6.07 \pm 3.3	2 \pm 1.47	9.52 \pm 3.63	42.62 \pm 24.81	48.12 \pm 29.31
B	3.44 \pm 2.71	8 \pm 1.52	10.82 \pm 2.75	18.6 \pm 6.51	19.90 \pm 7.19

*median, mean \pm S.D., n=6**Fig. 3** Plasma concentration- time curves of TE and marketed product after oral administration of marketed product (treatment A) and prepared formulation (treatment B)

Needless to say, marketed product tablets represent a sustained release dosage form. Yet, the designed chronotherapeutic system displayed a pulsatile drug release pattern.

The maximum drug concentration (C_{max} ; 6.07 ug/ml) of oral sustained release marketed tablets was achieved at a median T_{max} of 2 h. A lower C_{max} value (3.44 ug/ml) was estimated at a median T_{max} of 8 h for the designed chronotherapeutic system. The analysis of these values revealed statistically significant differences at $P < 0.05$. Following a T_L of 6 hours, TE was completely released within two hours. The prompt pulsatile delivery of TE could be concluded.

It is worth to note that no significant differences ($P > 0.05$) were observed between $AUC_{0-\infty}$ values of both treatments.

Conclusions

This study confirms that the developed system promoted the pulsatile delivery of a sparingly-soluble model drug without affecting the extent of its absorption. Further studies are necessary to evaluate the potential of this pulsatile system for water soluble and practically insoluble drugs.

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تقييم الاتاحة الحيويه لنظام دوائي مرتب زمنيا محمل بعقار مضاد للربو لتوصيله عن طريق الفم

للسادة الدكتورة

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^ب قسم الصيدلانيات و الصيدلة الصناعية- كلية الصيدلة – جامعة القاهرة

مع أنه يمكن لنظام العلاج النابض عن طريق الفم أن يعمل على تحرير الدواء في الوقت الصحيح لذروة المرض إلا أن عدم مراعاة المكان المناسب لامتصاص العقار قد يؤدي الى ضعف أو عدم امتصاصه على الإطلاق ولا سيما بالنسبة للعقاقير التي يتم امتصاصها بشكل تفضيلي من الجهاز الهضمي العلوي. أدت هذه الاعتبارات إلى تطوير أنظمه علاج حفظ معدى نابضه تعمل على حفظ العقاقير التي لديها نافذة محدوده للامتصاص في الجهاز الهضمي العلوي مما يساعد على تحسين الاتاحة الحيويه والنتائج العلاجية اللاحقة. أثبتت دراسات تقييم الاتاحة الحيويه لعقار الثيوفيلين اللامائي من أفضل الصياغات المحضرة للأقراص داخل الكأس بالمقارنه مع الأقراص التجاربه الممتده المفعول في متطوعين ذكور أصحاء صائمين وذلك عقب تناولهم جرعات من العقار عن طريق الفم.