Drug Release and Pharmacokinetics Behavior of a Simple Ethylcellulose Coating Pulsatile Tablet of Time-Controlled Explosive System

Mohui Yang¹, Xingli Wang², Jiabi Ouyang¹, Zhen Zhang¹, Yani Tan¹, Sha Li¹,³,⁴*

Author affiliations
¹College of Pharmacy, Jinan University, Guangzhou, China
²Shenzhen Polytechnic, Shenzhen, China
³International Cooperative Laboratory of Traditional Chinese Medicine Modernization and Innovative Drug Development of Chinese Ministry of Education, College of Pharmacy, Jinan University, Guangzhou, China
⁴Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Guangzhou, China

Author contribution:
* Sha Li is corresponding author of this paper.
†These authors contributed equally to this work.
Sha Li conceived the idea, designed and supervised the research. Mohui Yang and Xingli Wang performed the research, conducted the analysis and wrote the paper. Jiabi Ouyang, Zhen Zhang and Yani Tan contributed to data collection and analysis. All authors contributed to the writing and revisions.

Address reprint requests to
Sha Li
College of Pharmacy, Jinan University, Guangzhou 510632, China
Email: tlisha@jnu.edu.cn

Core tip:
The time rhythm of the human body is associated with the occurrence and development of many diseases. Kinds of diseases of particular onset biorhythm provided the room for the development of chronopharmacological drug delivery systems. Metoprolol tartrate (MT), a medicine commonly used for treatment of cardiovascular diseases in clinic, was selected as model drug to develop a time-controlled explosive pulsatile tablet. The prepared MT pulsatile tablets showed a drug release lag time of 7.10 h in vitro perfectly consistent with the absorption lag time of 7.04 h in vivo, which ensured the pulsatile tablet a promising chronopharmacological drug delivery system for MT to prevent and treat the peak attack of hypertension and angina pectoris early in the morning.

Abstract:
Background The time rhythm of human body is associated with the occurrence and development of many diseases. Kinds of diseases of particular onset biorhythm provided the room for the development of chronopharmacological drug delivery systems.
Aim In this work, the drug release and pharmacokinetics behavior of metoprolol tartrate (MT) pulsatile tablet developed in our lab was investigated to figure out its feasibility of convenient drug taking to exert effective chronotherapy for cardiovascular diseases like hypertension and angina pectoris.
Methods The in vitro release behavior of MT pulsatile tablets was investigated by using basket method. The appearance and morphology of MT pulsatile tablets during drug release was observed by naked eye and
scanning electronic microscope, respectively. In vivo pharmacokinetics performance was studied in New Zealand rabbits.

**Results** The lag time of MT pulsatile tablets was approximately 7 h in vitro, and a fast release was observed thereafter, with more than 90% releasing within 10 min. The pharmacokinetics study in rabbits demonstrated a perfect consistence in the absorption lag time of 7.04 ± 0.29 h in vivo. Compared with the marketed conventional tablet, the MT pulsatile tablet showed a bioequivalence in absorption extent with a relative bioavailability of 110.04%, but not in absorption rate.

**Conclusion** The designed lag time of 7 hours enabled the MT pulsatile tablets to achieve effective chronotherapy for cardiovascular diseases like hypertension and angina pectoris with a high attack rhythm around 4:00-6:00 A.M by giving medicine conveniently around 22:00 P.M. the night before.

**Key words** Pulsatile tablets; Metoprolol tartrate; Drug release; Pharmacokinetics; Bioequivalence.

**INTRODUCTION**

In recent years, the biological rhythm, generally circadian rhythm, of the occurrence and severity of some diseases has been better known by chronopharmacological and chronobiological study. The rhythm was obviously observed in the manifestation and severity of some symptoms of chronic diseases, such as hypertension, allergic rhinitis, asthma, duodenal ulcer and arthritis, and even in the onset of some serious life-threatening medical events, like myocardial infarction and angina pectoris. In order to prevent the onset of such fatal medical events and to improve the treatment outcome of these diseases, chronotherapy was tailored to synchronize drug application in accordance with the natural rhythm of the body and the diseases.

The demand of chronotherapy promoted the development of chronopharmacological drug delivery system. To date, the studies of pulsatile drug delivery generally employed tablets, capsules or multiparticulate formulations with swellable, erodible, insoluble, soluble, rupturable or permeability changeable coatings, capsules with different types of release-controlling plugs, and osmotic pump technique as well. Also, some particular stimuli, such as glucose concentration, pH, temperature, magnetic field, ultrasound, electric field, light and mechanical force was studied to trigger the pulse release of drug from hydrogels, polymeric micelles or microspheres. Nowadays, several chronopharmacological technologies have already been on the market and available for treatment of morning or early morning hypertensive management and asthma control, like Cardizem®LA, Covera®HS, InnoPran®XL, Uniphyl® and Verelan®PM.

In the kinds of chronopharmacological drug delivery systems, time controlled explosive system (TES) was commonly adopted in pulsatile drug delivery studies and of feasibility for scale-up manufacture. In our lab, we developed a pulsatile tablet of TES type with relatively simple structure and process by using metoprolol tartrate (MT), which was widely used for cardiovascular diseases treatment, as a model drug. It consisted of a core tablet and a coating layer of ethylcellulose (EC) prepared simply by direct compression and pan coating, respectively. The design objective was to achieve a lag time of 6-8 h and a fast complete pulse release of MT thereafter. Therefore, the MT pulsatile tablets may be taken at night around 20:00-22:00 P.M. and release at 4:00-6:00 A.M. next morning to match up the high attack of hypertension and/or angina pectoris in the early morning. The drug release in medium and pharmacokinetic behavior of MT pulsatile tablets in rabbits was investigated to evaluate the consistence of pulse release of drug in vitro and in vivo. Also, the pharmacokinetic characteristics of core tablets and marketed conventional tablets of MT were studied for comparison to access the bioequivalence.

**MATERIALS AND METHODS**

**CHEMICALS**

Metoprolol tartrate (MT) was supplied by Shandong Qihe Jinzun Chemical Co. Ltd. (Lot: 1001900, Shandong Province, China). Metoprolol tartrate reference standard was supplied by National Institutes for Food and Drug Control (Lot: 100084-200101, China). Phenytoin sodium

VOL 10, ISSUE 03, YEAR 2020 58
was obtained from Guangdong Taishan Xinning Pharmacy Co. Ltd. (Lot: 090202, Guangdong Province, China). Cross-linked sodium carboxymethyl cellulose (CC-Na), cross-linked polyvinylpyrrolidone (PVPP), microcrystalline cellulose (MCC), hydroxypropyl cellulose (HPC), aerosil and magnesium stearate were supplied by Shanghai Houcheng Fine Chemical Co. Ltd. (Shanghai, China). Ethyl cellulose (T7) was obtained from Guangdong Shantai Cellulose Co. Ltd. (Guangdong Province, China). Diethyl phthalate was obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Methanol was chromatography grade and water was redistilled water. All other chemicals were analytical grade. Conventional metoprolol tartrate tablets (25 mg/tablet) manufactured by AstraZeneca Pharmacy Co. Ltd. (Lot: 1003035, London, England) were obtained commercially. MT (25 mg/tablet) pulsatile tablets and core tablets were prepared in our lab.

**ANIMALS**

New Zealand rabbits (male, 2.5 ± 0.2 kg) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). The animals were housed in an environmentally controlled breeding room with free access to standard laboratory food and water. All animals were kept 7 days for acclimation before experiments. All the animal studies described in this work were approved and conducted in accordance with the guidelines of Laboratory Animal Ethics Committee of Jinan University.

**DRUG RELEASE TEST IN VITRO**

The drug release test of pulsatile tablets was carried out using basket method in 500 mL of simulation medium of gastrointestinal tract at 37 °C ± 0.5 °C and a rotation speed of 100 rpm (n = 6). In simulation medium of gastrointestinal tract, 0.1 mol/L HCl solution was used as release medium for the first 2 h, and then the medium was converted into PBS of pH 6.8 for the subsequent test. At certain time interval, the sample was withdrawn and analyzed by ultraviolet visible spectrophotometry to detect absorbance at a wavelength of 274 nm. The lag time was determined as the time point when the coating layer was visually observed ruptured. The dissolution behavior of core tablets and marketed conventional MT tablets was investigated as well for comparison. Furthermore, the influence of medium temperature, rotation speed and medium sort on lag time and drug release behavior of MT pulsatile tablets was investigated. The appearance of pulsatile tablets before and during drug release test was photographed, and the morphology change of both coating film and core tablets was observed by using scanning electronic microscope (SEM, Philips, the Netherland).

**PHARMACOKINETICS STUDY IN RABBITS**

Pharmacokinetics study was carried out in New Zealand rabbits. The pharmacokinetics behavior of MT pulsatile tablets was evaluated, and that of MT core tablets and marketed conventional MT tablets (25 mg/tablet, AstraZeneca Pharmacy Co. Ltd., London, England) was studied as reference. Six New Zealand rabbits (male, 2.5 ± 0.2 kg) were randomly divided into three groups and fasted with free access to water for 24 h before dosing. The rabbits were given MT pulsatile tablets, MT core tablets and marketed MT conventional tablets, respectively, 2 tablets (50 mg) per rabbit. The study was performed by a cross over design of 3 cycles and 7-day washout period. The blood samples (1 mL) were withdrawn via ear vein before dosing (0 h) and at 0.08, 0.16, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 10 h for marketed tablet reference group and core tablet group, or at 2, 4, 6, 6.25, 6.5, 6.75, 7, 7.25, 7.5, 7.75, 8, 8.5, 9, 11, 13 and 15 h for pulsatile tablet group, respectively. The blood sample was centrifuged at 4000 rpm for 5 min and the collected plasma was stored at -20 °C until assayed.

Metoprolol tartrate in the plasma was assayed using a reversed-phase high-performance liquid chromatography (RP-HPLC) method. Briefly, the plasma sample (500 µL) was added with internal standard solution of phenytoin sodium (24 µg/mL, 20 µL) and sodium hydroxide solution (1 mol/L, 500 µL) followed by extraction of ethyl acetate (3 mL). The organic extracts
were separated and dried under a nitrogen flow at 37 °C, and then reconstituted in mobile phase. After a centrifugation at 12000 rpm for 5 min, 20 µL of supernatant was injected into phenomenex C18 column (250×4.6 mm, 5 µm). The mobile phase consisted of methanol and water phase (containing 1.6 mL triethylamine and 0.5 mL phosphoric acid in 385 mL water) (60:40). An isocratic flow rate was set at 0.8 mL/min, and the wavelength of detection was 223 nm. The data were processed by DAS 2.1.1 software (Chinese Mathematical Pharmacology Society) and pharmacokinetic parameters were calculated by non-compartmental model.

STATISTICS
Data were statistically evaluated using one way ANOVA by SPSS 13.0 software. P value less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION
DRUG RELEASE BEHAVIOR IN VITRO
The drug release curves of marketed conventional tablets, prepared pulsatile tablets and core tablets in vitro were illustrated in Fig. 1A. The prepared MT pulsatile tablets showed a typical sigmoid drug release curve with a lag time about 7 h. The absorbance of MT at 274 nm was detected as soon as the coating layer ruptured at the lag time. The drug released fast with about 90% entering the release medium within 10 min after lag time. Both the core tablets and marketed conventional tablets released MT quickly and completely, and the core tablets released somewhat faster. The drug release behavior of core tablets and pulsatile tablets after lag time was much similar.

As shown in Fig. 1B-D, under different drug release environments, the MT pulsatile tablets all exhibited typical pulse release characteristics. The lag time in drug release profiles was about 7 hours and there was no obvious fluctuation, indicating that the temperature, rotating speed and different pH values of the drug release environment would not have a significant impact on the lag time. Only the rotation speed had a slight effect on the drug release rate, but the fluctuation was small, and the accumulative release percentage fluctuated within 10 minutes was only about 10% (50 ~ 150 rpm), and the temperature and type of release mediums almost had no effect on it. This was because ethylcellulose was a controlled-release coating material insoluble in the physiological pH range of the entire human gastrointestinal tract, so changes in the pH value of dissolution medium had little impacts on its permeability and drug release profiles. The different rotation speed might create different degree of hydrodynamics which might affect the initial speed at which the core tablet dispersed outside of the film at the beginning of film rupture. However, the core of the pulsatile tablets had good swelling and dispersing properties. Therefore, although the accumulative release percentage fluctuated within 10 minutes after lag time, the fluctuation was fairly small. With time prolonged to 30 minutes after lag time, the fluctuation was quickly compensated by the good swelling and dispersing properties of the core tablet and was almost completely disappear, and the drug release was greater than 95%. In general, the developed MT pulsatile tablets showed good performance within the range of human body temperature fluctuation, gastrointestinal motility difference and gastrointestinal tract pH change. The lag time of drug release remained basically the same, and the drug could release quickly and completely after the designed lag time due to the good disintegration and dissolution properties of the tablet core.
Drug Release and Pharmacokinetics Behavior of Ethylcellulose Coating Pulsatile Tablets

Figure 1 Drug release profiles of pulsatile tablets, core tablets and marketed conventional tablets of MT. A described the MT release behavior from pulsatile tablets, core tablets and marked conventional tablets. B-D described the influence of temperature, rotating speed and medium sort of drug release test on MT release from pulsatile tablets, respectively. Q% was the accumulative drug release percentage.

APPEARANCE AND MORPHOLOGY OF PULSATILE TABLETS DURING DRUG RELEASE
Before the rupture of coating layer, the appearance of MT pulsatile tablets kept same as that before test and the thickness of tablets kept unchanged as well. At lag time, small crack was formed and the coating film bursted quickly like an open shell with the core tablet expanding and dispersing out within 4 min (Fig. 2A-D). The SEM micrographs of MT pulsatile tablets before and during drug release test were showed in Fig. 2E-I. No matter in the transection or surface micrographs, no obvious change was observed in the structure of coating layer of EC at 6 h after drug release and even if at lag time. However, the core tablet became compacted at 6 h due to the expansion caused by water uptake and then turned thoroughly puffy at lag time when contacting with water directly.

Figure 2 Appearance and morphology of MT pulsatile tablets before and during drug release test. A-D were photographs of appearance of pulsatile tablets before (A) and 435 min (B, at the lag time), 437 min (C), 439 min (D) after test. E-G were SEM micrographs of the transection of pulsatile tablets before (E) and 6 h (F) after test, and after the rupture of coating film (G). H and I were the SEM micrographs of the surface of coating layer of pulsatile tablets before test and after the rupture of coating film. 1-core tablet; 2-coating film.
PHARMACOKINETICS STUDY IN RABBITS
The mean plasma concentration-time profiles of different MT tablets were shown in Fig. 3. The marketed conventional tablets and the prepared core tablets exhibited no lag time of absorption, while the prepared pulsatile tablets showed a lag time of about 7.04 h followed by rapid increase of plasma concentration. The pharmacokinetic parameters of conventional tablets, core tablets and pulsatile tablets were summarized in Table 1. Pharmacokinetic parameters as $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$, $t_{1/2}$ and $C_{\text{max}}$ of pulsatile tablets showed no significant difference from those of conventional tablets and core tablets ($p>0.05$), but $t_{\text{max}}$ exhibited significant difference. This indicated that the pulsatile tablets significantly prolonged the lag time of drug release while not reduced the drug release rate and extent. When taking conventional tablets as reference, the relative bioavailability of pulsatile tablets was 110.64%, the 90% confidence interval range was 99.64%~116.88%, meeting bioequivalence standards (80~125%). This indicated that the absorption extent of MT was not influenced by the pulsatile tablets.

Table 1 Pharmacokinetic parameters of metoprolol tartrate pulsatile tablets, conventional tablets and core tablets (mean ± SD, n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conventional tablets</th>
<th>Core tablets</th>
<th>Pulsatile tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{AUC}_{0-t}$ (mg/mL·h)</td>
<td>7.37±0.66</td>
<td>6.58±0.58</td>
<td>8.21±1.43</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (mg/L·h)</td>
<td>7.93±0.64</td>
<td>7.01±0.54</td>
<td>8.91±1.48</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.31±0.25</td>
<td>2.39±0.63</td>
<td>2.16±0.45</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>0.75±0.00</td>
<td>0.75±0.00</td>
<td>7.92±0.20</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>2.81±0.39</td>
<td>3.59±0.53</td>
<td>2.91±0.58</td>
</tr>
</tbody>
</table>

CONCLUSION
A kind of pulsatile tablets of MT with a simple structure were designed to be given conveniently around 22:00 P.M. and release drug rapidly at 4:00~6:00 A.M. early next morning around the peak incidence time of hypertension and/or angina pectoris. The pulsatile tablets released drug in a manner similar to the marketed conventional tablets in the gastrointestinal tract after reaching the lag time. Also, after lag time of absorption, the pharmacokinetics behavior of MT pulsatile tablets was similar to that of conventional tablets, and MT pulsatile tablets were bioequivalent to conventional tablets in absorption extent. The results indicated that the developed MT pulsatile tablets were expected to realize effective prevention and treatment of the threatening onset of hypertension and/or angina pectoris early in the morning.
ACKNOWLEDGMENTS

This work was financially supported by projects of Science and Technology Program of Guangzhou, China (No. 201704020087 and No. 201704020198) and Natural Science Foundation of Guangdong Province (No. 2020A1515010990). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES


Article citation:

Conflicts of Interest: No conflict of interest.

Disclaimer: Any views expressed in this paper are those of the authors and do not reflect the official policy or position of the Department of Defense.

Source of funding: This work was financially supported by projects of Science and Technology Program of Guangzhou, China (No. 201704020087 and No. 201704020198) and Natural Science Foundation of Guangdong Province (No. 2020A1515010990). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.