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Full Length Research Paper

Preparation of carteolol hydrochloride matrix sustained-release pellets and evaluation *in vitro/in vivo*

H. Zhang, Y. H. Luo*, X. Y. Zhao, Q. Chen, M. Luo, L. Y. Luo, C. C. Tian, Z. K. Ge and Y. J. Zhang

College of Pharmaceutical Sciences, Southwest University, Chongqing, China.

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The aim of this study was to prepare carteolol hydrochloride matrix sustained-release pellets, and to compare its performance *in vivo* with carteolol common tablets. The formula was optimized according to the roundness and the release of pellets with single-factor, and orthogonal test. The carteolol hydrochloride matrix sustained-release pellets were got by extrusion-spheronisaion, while the release rate *in vitro* was determined by rotate basket method with UV detection. The carteolol hydrochloride sustained release property was studied in New Zealand white rabbits after oral administration. The optimized prescription is composed of carteolol hydrochloride 5.5 g, MCC 6.0 g, SA 26.3 g, EC 21.0 g, CMC-Na 1.2 g. Drug release property *in vitro* were as follows: the release time is over 12 h, the drug release behaviors follow the first order equation and the process of drug releasing is drug diffusion with frame erosion. The C_{max}, T_{max} and $AUC_{(0\rightarrow24)}$ of the pellets were 2.58 ± 0.43 (μg/ml), 4.20 ± 0.87 (h) and 22.19 ± 4.23 (μg/ml h), respectively. The C_{max}, T_{max} and $AUC_{(0\rightarrow24)}$ of the tablets were 3.75 ± 0.59 (μg/ml), 1.00 ± 0.72 (h) and 18.70 ± 3.75 (μg/ml h). The carteolol hydrochloride sustained-release pellets have reasonable formulation and simple preparation technology; the pellets acquired perfect sustained-release properties *in vitro/in vivo*.

Key words: Carteolol hydrochloride, sustained-release, pellets, *in vitro*, *in vivo*, evaluation

INTRODUCTION

Carteolol hydrochloride, a beta blocker with intrinsic sympathomimetic activity (Floreani et al., 2005; Floreani et al., 2008; Bruck et al., 2008), is used to treat hypertension, arrhythmia, angina, myocardial infarction, glaucoma (Henness et al., 2007) and other diseases. Carteolol hydrochloride also has been used for the treatment of cardiac neurosis (Knieling and Athanasiadis, 2005); congenital tetralogy of fallot (Apitz et al., 2009; Ohtsuka Pharmaceutical Co., 2008). Only two carteolol hydrochloride formulas are stated in pharmacopoeias. One is its eye drops in Chinese Pharmacopoeia, the other one is its common tablets in the United States Pharmacopeial Convention (USP). So far, the research reports about carteolol hydrochloride matrix sustained-release pellets has not been reported both in China and abroad, only the patent of carteolol hydrochloride matrix sustained-release pellets applied by our research group can be seen (Luo et al., 2010). Because of the shorter terminal elimination half-life of carteolol hydrochloride (1.73 to 2.08 h) (Morita et al., 1977), patients need multiple doses in one day. Therefore, it is useful to develop carteolol hydrochloride matrix sustained-release pellets, which have significant practical meaning with reducing the frequency of administration and improving patient medication compliance. We prepared carteolol hydrochloride matrix sustained-release pellets, and investigated the drug release properties of pellets *in vitro* and *in vivo*.

MATERIALS AND METHODS

Apparatus

E-50/CGC-350 Extrusion-spheronisation machine (Enger Granulating and Coating Technology Co. Ltd, Chongqing, China);

*Corresponding author. E-mail: luoyonghuang@126.com. Tel: +86 23 13883808688. Fax: +86 23 68251048.

HitachiU-1800 Ultraviolet spectrophotometer (Hitachi, Japan); RCZ-6B2 Drug dissolution instrument (Huang Hai Medicine Checking Instrument Co. Ltd, Shanghai, China); HPLC (Shimadzu LC-20AD, Japan).

Materials

Carteolol hydrochloride (99.57% purity, Lot 090320) was obtained from Beibei institute of modern drug application (Chongqing, China); carteolol hydrochloride sustained-release pellets used contained 15/164 mg, and were manufactured by our laboratory (Lot 20110709). Carteolol hydrochloride tablets (5 mg per tablet) obtained from Otsuka Pharmaceutical Co Ltd (Japan) were chosen as the reference; microcrystalline cellulose (MCC) was obtained from Shanhe Medicinal Auxiliary Materials Co. Ltd (Anhui, China). New Zealand white rabbits were obtained from Laboratory Animal Center of Chongqing Medical University; all other chemicals were of chemical grade.

Methods

Release rate determination of carteolol hydrochloride matrix sustained-release pellets in vitro

According to determination of carteolol hydrochloride tablets' dissolution in USP (USP30-NF25, 2007), rotating basket method was adopted to determine the carteolol hydrochloride matrix sustained-release pellets. Purified water was selected as the dissolution medium, rotation speed was 50 r/min and temperature was 37 ± 0.5°C, 10 ml dissolution medium was sampled in 1, 2, 4, 6, 8, 10 and 12 h after putting the self-made carteolol hydrochloride matrix sustained-release pellets into the basket, and 10 ml dissolution medium was added at the same temperature promptly after each sample. The sample solution was filtered through 0.45 μm microporous membrane. The absorbance was determined by UV spectrophotometry at 251 nm (standard curve equation: $A = 0.0271C + 0.0013$, $R^2 = 0.9999$). The absorbance of the carteolol hydrochloride tablets was determined with the same method, and the sample was taken in 0.5, 1, 2, 4, 6, 8, 15, 30, 45 and 60 min, respectively.

Single factor test

On the basis of preliminary experimental data, 10, 20 and 30% of MCC were used; the proportion of ethyl cellulose (EC) to stearic acid (SA) was 1:1, 1:1.5 and 1:2; the amount of sodium carboxymethyl cellulose (CMC-Na) was 1, 2 and 3%. The aforementioned experiments were performed in order to study the effects of different dosages of MCC, the different proportion of EC and SA, and different dosages of CMC-Na, on the pellets drug release behaviors *in vitro*.

Orthogonal test

According to the single factor test results, the content of MCC (A), the proportion of EC and SA(B), and the content of CMC-Na (C) were selected as the three factors, each factor had three levels, which was: A(%): 8, 10, 12; B:1:1.25, 1:1.50, 1:1.75; C(%)1, 2, 3. The orthogonal test was arranged as L9 (3^4) table.

Referring to the requirement about sustained-release preparations *in vitro* from USP22 version, the pellets roundness and release in main time points were selected as evaluation indexes. The drug cumulative release amount was investigated (P) in 2, 6 and 12 h , and the corresponding cumulative releases were 35 , 50 and 75% as standards, respectively (Hong et al., 2006; Cai et al., 2003). P = [P (2h) - 35%] + [P (6 h) - 50%] + [P (12 h) - 75%]. Referring to the preceding equation, the lower the P value, the closer it is from the standard. Angle of repose α which was measured by fixed-funnel method reflected the roundness of pellets (Dong et al., 2008). A smaller α value represents the better roundness of the pellets. The total score is calculated by the equation R = P + α.

The preparing of pellets

The extrusion-spheronisation method was used to prepare pellets (Vervaet et al., 1995; Dukic-Ott et al., 2009). According to the optimized formulation of carteolol hydrochloride matrix sustained-release pellets, we sieved the raw materials and excipients through the No.6 sieve respectively, and then mixed them. A wet material with purified water was prepared as wetting agent; extrusion speed was set as 45 r/min. The prepared wet material was extruded into strips (the screen aperture was 0.8 mm); The spheronisation speed was set as 750 r/min for 6 min, the extruded strips were prepared into pellets; prepared pellets were put into air drying oven, and dried at 55°C for 6 h, following drying at 80°C for 4 h.

Sustained-release property evaluation in vivo

Six New Zealand white rabbits which were composed of male and female in equal were divided at random into two groups. Throughout the experiment, rabbits were housed, one rabbit per cage, in a room temperature. The study was conducted according to a randomized 2-period crossover with a 1-week washout period between treatments design. The doses of pellets and common tablets were chosen as 164 mg (containing 15 mg carteolol hydrochloride) and 3 tablets (containing 5 mg per tablet), the oral dosage form was given with 10 ml water. Blood samples (2 ml) were collected into vacutainers (containing sodium heparin as anticoagulant) at 0.25, 0.5, 0.75,1, 2, 4, 6, 8, 10, 12 and 24 h after dosing. Following centrifugation, the plasma was then pipetted into polypropylene tubes and frozen immediately, stored at -20°C until analysis.

The plasma was precipitated with acetonitrile; the organic layer was transferred to a clean spiky bottom tube after centrifugation and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved with 200 μl of mobile phase and then centrifuged at 10000 (r/min) for 5 min to precipitate solid impurity of the residue. An aliquot of 20 μl of the resulting solution was injected into the HPLC system. A VP-ODS C_{18} column was used, the mobile phase consisted of acetonitrile and 0.0047 $molL^{-1}$ Na_2HPO_4 (13:87, V: V) (Ge et al., 2011). The flow rate was 1 ml/ min and the UV detector was set at 252 nm.

RESULTS

Single factor test

The results showed that when the content of MCC was 10%, the drug release rate was the slowest (Figure 1). Considering with both pellets forming properties and drug release rate, the content of MCC was preliminarily determined as approximately 10%. The drug release rate of the pellets with the proportion of EC and SA (1:2) was the slowest, but the pellets with this proportion would stick together when they were heated at 80°C. So, the

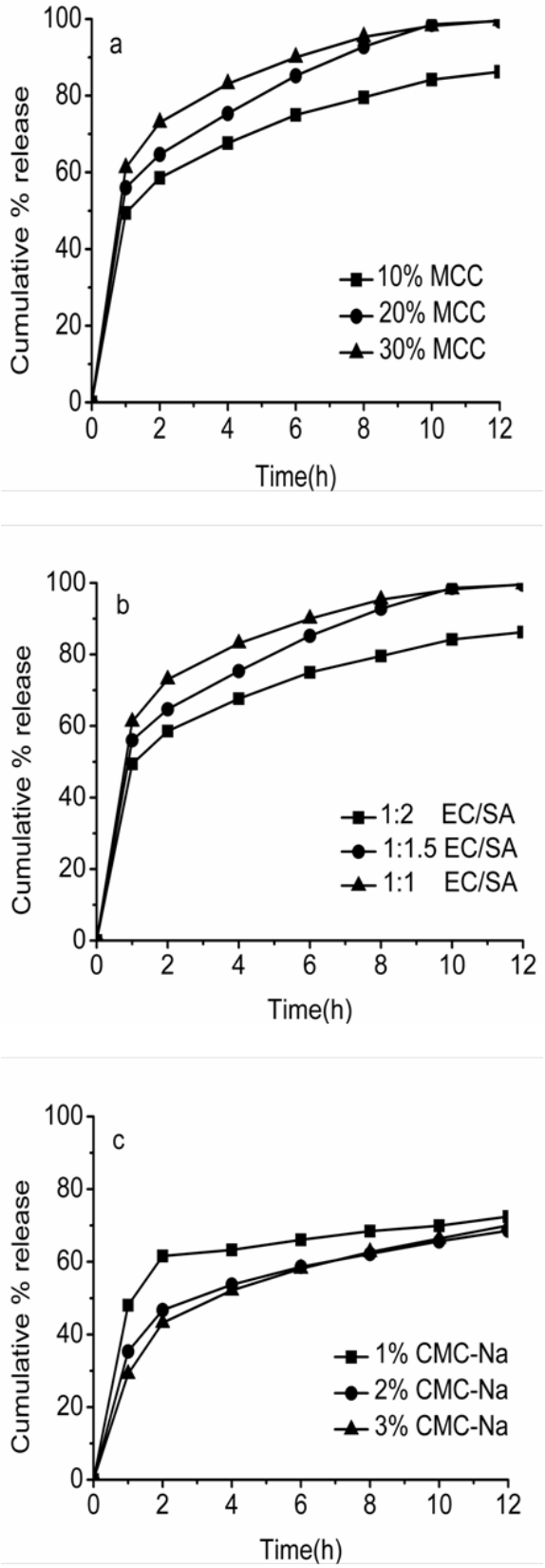

Figure 1. The release profiles of carteolol hydrochloride from the sustained-release pellets. a) The amounts of MCC; b) the proportion of EC and SA; c) the amounts of CMC-Na.

proportion was identified as about 1:1.50 initially. The drug release rate was significantly reduced when the content of CMC-Na was 3%, especially in the early stage of drug release (Figure 1).

Orthogonal test

According to the results of range analysis, the order of various factors influencing the roundness and release degrees of the pellets was: C > A > B. At the optimal level of each factor, the optimal prescription of the sustained-release pellets was $A_2B_1C_2$, which means that the content of MCC was 10%, the ratio of EC/SA was 1: 1.25, the content of CMC-Na was 2% (Table 1).

The formulation of the pellets and the drug release profile of pellets *in vitro*

According to the results of the single factor and the orthogonal test, the prescription of the pellets was composed of carteolol hydrochloride 5.5 g, MCC 6.0 g, SA 26.3 g, EC 21.0 g, CMC-Na 1.2 g. The pellets were prepared by the extrusion-spheronisation method. Referring to the release rate determination method, the drug release was determined. The present results showed that the sustained-release pellets possessed obvious sustained release effects comparing with the carteolol hydrochloride tablets (Figure 2).

Drug release model fitting *in vitro*

The release data of the pellets was fit with zero order kinetics equation, first-order kinetics equation, Higuchi equation and Peppas equation (Costa and Lobo, 2001; Gil et al., 2006). The results showed that zero order kinetics equation: $Q = 5.801t + 19.30$, $r = 0.9024$; first-order kinetics equation: $Q = \ln (100 - Q) = -0.134t + 4.501$, $r = 0.9816$; Higuchi equation: $Q = 27.16t^{1/2} - 8.377$, $r = 0.9721$; Peppas equation, $LnQ = 0.704lnt + 2.784$, $r = 0.9471$, $n = 0.704$ ($0.43 < n < 0.85$).

Sustained-release property

The pharmacokinetics of carteolol hydrochloride matrix sustained-release pellets and carteolol hydrochloride common tablets were investigated. The plasma concentration versus time curves for the pellets and tablets are shown in Figure 3, the pharmacokinetic parameters (C_{max}, T_{max}, and AUC_{0-24}) are given in Table 2.

DISCUSSION

MCC which was prepared by following the prescription

Table 1. Results of orthogonal test.

No.	A	B	C	P	α	R
1	1	1	1	50.19	22.4	72.59
2	1	2	2	10.98	23.6	34.58
3	1	3	3	79.39	31.2	110.59
4	2	1	2	8.92	25.7	34.62
5	2	2	3	69.25	28.0	97.25
6	2	3	1	45.73	24.5	70.23
7	3	1	3	73.00	27.6	100.6
8	3	2	1	74.09	20.3	94.39
9	3	3	2	54.28	30.4	84.68
I	217.76	207.81	237.21			
II	202.1	226.22	153.88			
III	279.67	265.5	308.44			
I_1	72.59	69.27	79.07			
II_2	67.37	75.41	51.29			
III_3	93.22	88.50	102.8			
r	25.85	19.23	51.51			

A: the content of MCC; B: the proportion of EC and SA; C: the content of CMC-Na; P: the drug cumulative release amount; α: angle of repose; R = P + α; r: the value of range analysis.

formed fracture and pore canal both on the surface of pellets and interior after absorbing water and expanding in the dissolution media. Meanwhile, the dissolvent infiltrated into pellets, which dissolved their internal structures, producing a concentration gradient to make the drug release constantly. EC and SA in the prescription were blockers. SA formed a dense structure around EC after heated, which contributed to the slow release of the drug. EC played the role of strengthen when it is embedded in the corrosion skeleton formed by SA. Meanwhile, EC blocked some pores to increase the curvature of the pore canal, so that it delayed the release rate of the drug (Chen et al., 2003; Ahmed and Souad, 2008; Akhlaq et al., 2011). When preparing water soluble drugs such as carteolol hydrochloride into matrix sustained-release pellets, the biggest problem was how to avoid the burst problem in the early drug release stage. The problem was solved in the present study by adding a certain amount of CMC-Na into the prescription. CMC-Na possessed better water affinity and faster hydrating rate, which made the gel layer form quickly and controlled drug release (Liu et al., 2007). Further study is needed to prove whether CMC-Na and other excipent could solve the early release problems for water soluble drugs.

The release behavior of carteolol hydrochloride matrix sustained-release pellets we prepared was in accordance with the first-order kinetic process, which suggested that the drug release rate *in vitro* was in direct proportion to drug concentration. Peppa equations obeyed the exponential law. It is a semi empirical equation about integrated diffusion and dissolution, which could interpret

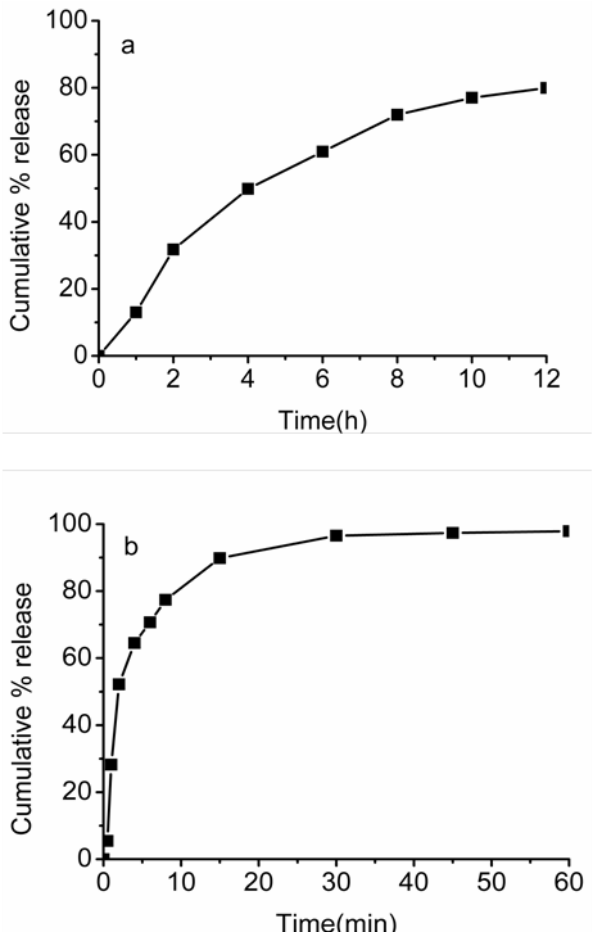

Figure 2. The release profiles of carteolol hydrochloride from the sustained-release pellets, a: carteolol hydrochloride matrix sustained-release pellets; b: carteolol hydrochloride common tablet.

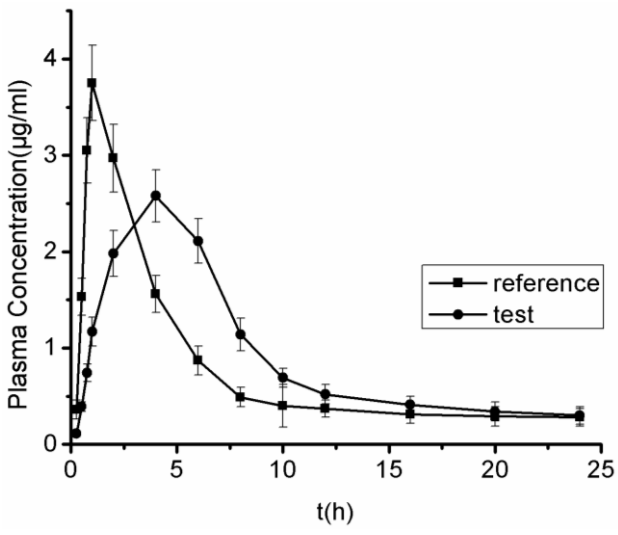

Figure 3. Mean plasma carteolol hydrochloride concentration-time curves of carteolol hydrochloride matrix sustained-release pellets and the tablets.

Table 2. Pharmacokinetic parameters of test and reference preparations.

Variable	T_{max} (h)	C_{max} (μg/ml)	$AUC_{(0\rightarrow24)}$ (μg/ml h)
Test	4.20 ± 0.87	2.58 ± 0.43	22.19 ± 4.23
Reference	1.00 ± 0.72	3.75 ± 0.59	18.70 ± 3.75

All the data are presented in the form of mean ± S.D.

the mechanism and process of drug release. The equation is $Mt/M\infty = kt^n$, Mt and M∞ represent the cumulative release amount at t time and infinity point, respectively, k is the constant of skeleton structure and geometrical characteristics, n is the release index. For the spherical matrix drugs like pellets: when $n \leq 0.43$, the drug release mechanism is Fickian diffusion; when $0.43 < n < 0.85$, the drug release mechanism is non-Fickian diffusion; when $n \geq 0.85$, the drug release mechanism is frame erosion. Fitting the process of carteolol hydrochloride matrix sustained-release pellets releasing *in vitro* by Peppas model, n = 0.704, the data showed that the drug release mechanism of the matrix sustained-release pellets was drug diffusion accompanied with frame erosion.

The plasma level of carteolol hydrochloride tablets rose quickly and the maximum concentration (3.75 μg/ml) was reached 1 h after administration. There was a marked fall in plasma concentration between 1 and 12 h; for the pellets, the maximum concentration (2.58 μg/ml) was reached 4 h after administration, and the drug concentration fell slowly between 4 to 12 h. The time of drug concentration over the effective blood drug concentration (0.52 μg/ml) of pellets and tablets were 11.25 and 5.50 h, respectively. It was clear that the behaviors *in vivo* of the carteolol hydrochloride matrix sustained-release pellets were much better than the tablets.

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