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Different pH-values of Release Medium Influence the Drug release from PTX-PCL Microspheres

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Abstract: In this paper we study in vitro dissolubility of paclitaxel-loade polycaprolactone sustained-release microspheres. Different pH values release medium of is used to simulate pH conditions in different parts of body, and determination the paclitaxel in Microspheres by High Performance Liquid Chromatography according Chinese Pharmacopoeia 2010. The experimental results indicate that the microspheres release rates of same drug loading content in buffer solution of pH 7.35 is the fastest, and in the pH 1.2 is the slowest. The drug release behavior according to the first-order model and it is not affected by drug loading rate of microspheres. The prepared paclitaxel-loade polycaprolactone sustained-release microspheres has good sustained release effect in different release media, and the results can provide references for further study of in vivo release.

Keywords: Different pH-values, paclitaxel, polycaprolactone, microspheres

1. Introduction

Paclitaxel (PTX) is a new and effective broad-spectrum anticancer drug and has good therapeutic effect on breast cancer, ovarian cancer, gastric cancer and other tumors [1]. Nowadays injection is mostly used in clinical, and it is administrated after resolving polyoxyeylenated castor oil in ethanol, but due to toxic and side effects are caused after administration, such as severe allergic reactions and neutropenia [2]. Therefore, the develop--ment of safe, biocompatible and biodegradable drug carrier for PTX-loading has great significance on clinical application. It is determined in the in vitro release experimental that paclitaxel-loade polycapro--lactone sustained-release microspheres (PTX-PCL-MS) has good slow release property. Appropriate in vitro release test can effectively reflect the in vivo release of microspheres, in favor of selecting better prescriptions and technique.

Microsphere is a spherical or similar particle made of appropriate polymer material as the carrier to entrap or adsorb drugs, it is the recently developed new drug administration system, it can not only protect drugs from damage, but also have a special affinity with some cells and tissues, and it can be swallowed by reticuloendothelial system of organs and tissues and fused by cells, to concentrate in the target area, spread gradually and release drugs, or to be degraded by enzymes in lysosomes and release drugs, so microsphere is a passive targeting preparation in clinical treatment. In addition, during microsphere preparation, magnetic particles are entrapped in it to make magnetic microspheres, and in vitro orientation is achieved by using two-dimensional magnetic field, so it enables its active targeting ability. Microsphere has many routes of administration, including subcutaneous, intravenous and intramuscular injection, chamber administration, oral administration, etc[3]. In this study, the dynamic dialysis method[4] is adopted to study in vitro release in media of different pH respectively, and mathematical model is established to investigate the law of in vitro drug release of microspheres. The study on the release of microspheres in different environment provides the basis for clinical application and makes the best play of drug efficacy.

2. Experiments

2.1. Preparation of PTX-PCL-MS

We prepared PCL microspheres by dialysis method, weigh a certain amount of PCL to dissolve in organic solvent, stir at constant speed at 30 ℃ to fully dissolved, then add a certain amount of synperonic pe(R) (F68) to mix uniformly, and finish preparing the organic solvent solution containing F68 biodegradable polymers. We continue stirring at the same temperature and speed, use dynamic injection titrator to take 1mL above-mentioned solution to sustainedly drop into 10mL deionized water in 2h, and form the emulsion entrapped with PTX. After dropping, we continue stirring for 2h. We move the above-mentioned emulsion into the dialysis bag

(MWO=8000-14000), place into 1L deionized water for dialysis to remove the organic solvent, replace the water every 6 hours for three consecutive days, and get the blank aqueous dispersion of microspheres. After dialysis, we centrifuge and disperse the emulsion in the dialysis bag and take the sediment for freezing and drying.

2.2. Fabrication of Standard Curve

2mg PTX control is dissolved in 2mL chromatographic-grade methanol to prepare 0.1mg/mL control solution, and 0.5mL solution is placed into 25mL volumetric flask, which is diluted with chromatographic-grade methanol to scale, to prepare 0.02mg/m control solution. The control solution is diluted with methanol gradiently, to get the control solutions of various concentrations, 20μL control solutions are injected into high efficiency liquid chromatograph respectively to measure their peak areas and calculate results, and get the regression equation: A =-3.210+29.687C, r=0.9986 (n=6), presenting good linear relationship in the range of 0.050~0.50μg.

2.3. In Vitro Release of Microspheres

We weigh certain amount of drug-loaded polymeric micelle, add appropriate amount of water for dissolving, and transfer 2 ml into the dialysis bag of 14,000 MWCO. We immerse the dialysis bag in a certain amount of release medium, such as 30ml phosphate buffer solution (PBS) , and make the in vitro release experiment under magnetic stirring at 37℃. Every 2h, the release medium is replaced with fresh medium, by taking 20mL release solution and adding 20mL fresh PBS, i.e. the replacement volume is 20 mL. In order to investigate the influence of release medium on drug release, phosphate buffer solutions of pH 7.35, 6.5 and 1.2 are selected as the release media. In need of design for blank control, this study measures the release of PTX material in different pH conditions.We adopt HPLC detection to get the PTX content in the release solution, and then calculate the cumulative release volume. The formula is：

$$Er = \frac{Ve\sum_{1}^{n-1} Ci + VoCn}{m_{drug}} \qquad (1)$$

In the formula: Er is the cumulative release volume of PTX; Ve is the release solution replacement volume, 20mL; Vo is the volume of the release solution, 30mL; Ci is the concentration of the drug released at the i-th replacement, μg/mL; mdrug is the PTX weight in the drug-loaded micelle used for release, μg; n is the number of release solution replacement.

3. Study on drug release behavior

In this study, three microspheres with encapsulation ratios of 35.20% (M1), 50.68% (M2) and 68.30% (M3) are selected to test the release in different pH value buffer solutions, and the release curve is shown in Figure 1.

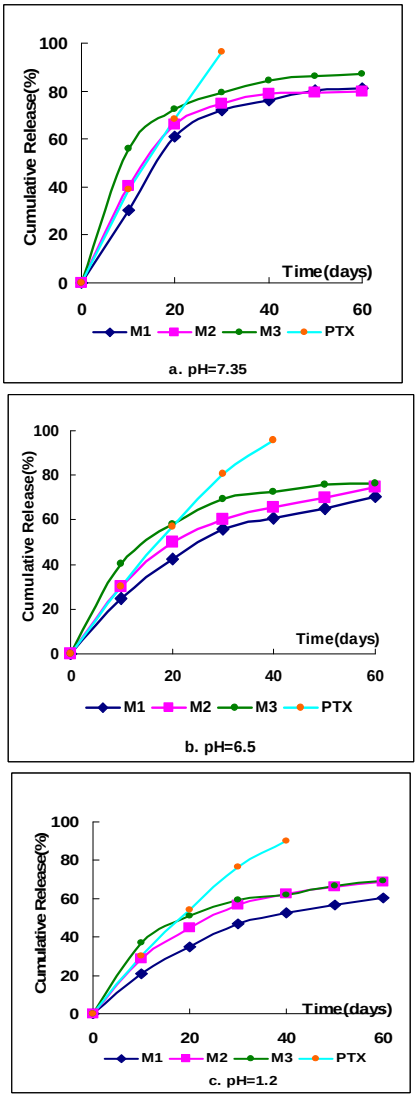

Figure 1. Release curve of PTX material and PTX-PCL-MS in different pH values release medium

It can be drawn from the release curve, PTX material is rapidly released in different release media, while PTX-PCL-MS is slowly released in different release media, with the release rate reaching 40%~50% in about 20 days. The drugs reach the plasma drug concentration requirement within certain time. Drug release is completed in about 60 days, with a good sustained release property.

It can be seen in Figure 2 that in the same medium pH condition, the order of burst release is M3>M2>M1, and the encapsulation ratio shows severe burst release. It can also be seen from Figure 2 that the release rate of the microspheres of same entrapment rate decreases with pH, and burst release slows down.

It can be seen from Figure 2 that along with the increase of drug entrapment rate, the release time of microspheres also increases, and those with higher drug loading rate reaches 40% of drug release faster. The

release time increases to about 60D from 45D, not influenced by medium pH. In condition of same medium pH, the order of sustained release is M1>M2>M3.

3.2. In vitro release model fitting

Linear regression is adopted to make in vitro PTX-PCL-MS release model fitting according to Level 0 and 1 kinetic equation and Higuichi equation. The release of PTX-PCL-MS in buffer solutions of Ph7.35, pH6.5 and pH 1.2 is more in line with Level 1 drug release. The fitting equation of the release curve for the three drug-loading microspheres in buffer solutions of different pH is shown in Table 1.

Table 1. Fitting releasing curves equation of microspheres

Ph values	Microspheres	first-order equation	r
7.35	M1	Y=-0.0172 t+6.7871	0.9978
	M2	Y=-0.0236 t+6.5111	0.9767
	M3	Y=-0.0236 t+6.5111	0.9778
6.5	M1	Y=-0.0172 t+6.7871	0.9978
	M2	Y=-0.0172 t+6.7871	0.9978
	M3	Y=-0.0172 t+6.7871	0.9978
1.2	M1	Y=-0.0172 t+6.7871	0.9978
	M2	Y=-0.0172 t+6.7871	0.9978
	M3	Y=-0.0172 t+6.7871	0.9978

3.3. Dissolution curve evaluation by similarity factor

Fitting factor can evaluate the two curves for in vitro drug release, and similarity factor (f_2) can quantify and compare the similarity of solid preparation dissolution curves, which is recommended by FDA as a preferred method to compare the similarity of two dissolution curves. Basic equation is:

$$f2 = 50\lg\{[1+(1/n)\sum_{i=1}^{n} Wt(\bar{X}_{ii}-\bar{X}_{ri})^2]^{-1/2} \times 100\} \quad (2)$$

In the formula, f_2 is similarity factor, $\bar{X}_{ii}$ is percentage of cumulative release of the reference agent in time t, $\bar{X}_{ri}$ is percentage of cumulative release of the test agent in time t, n is the number of sampling, Wt is the weight.

When f_2 is among 50~100, the two in vitro release behaviors has no significant difference; when f_2 is more close to 100, the higher the degree of similarity. The weight at each time point is 1, according to the above formula, the release curves obtained by various methods are pairwise fitted, to calculate the similarity factor f_2. It can be find that PTX-PCL-MS in various release media, f_2 is among 73.95~87.09, higher than 50, indicating that the release of PTX-PCL-MS in various release media is similar, and the difference is not significant. While PTX material and PTX-PCL-MS are in various release media, the similarity factor f_2 between any two curves of the four release curves is 18.43~20.06, lower than 50, so it can be concluded that there is significant difference between PTX material and PTX-PCL-MS in vitro release curves, indicating that PTX-PCL-MS improves the dissolution of PTX and achieves a good sustained release effect.

Conclusion

In addition to dynamic dialysis method, the measurement of in vitro drug release can also adopt membrane diffusion method, sample separation method, continuous flow method, reverse dialysis technology, etc. This study adopts the classic dynamic dialysis technique to measure PTX-PCL-MS in vitro release with buffer solutions of different pH as the release media, and the results are satisfactory.

The study is made on PTX-PCL-MS in vitro release in different pH conditions, which is based on pH values in different parts of body, closer to the in vivo drug release condition, so as to accurately evaluate the drug release property. The study shows that the release of microspheres of same drug loading rate in buffer solution (pH 7.35) is the fastest, completed in about 45D. The release in hydrochloric acid solution (pH 1.2) is the slowest, completed in about 60D. Phosphate buffer solution of pH 7.35 simulates human body environment, buffer solution of pH 6.5 simulates intestinal fluid, and hydrochloric acid solution of pH 1.2 simulates gastric juice, so it can be drawn that PTX-PCL-MS releases in human body is the fastest, the release in intestinal fluid is slower, and the release in gastric juice is the slowest.

In order to present PTX-PCL-MS release, its release curves are fitted with drug release model in Level 0 and 1 equation and Higuchi equation. PTX-PCL-MS release in buffer solutions of pH 7.35, pH 6.5 and pH 1.2 is more in line with Level 1 drug release. The release is not influenced by drug-loading rate of microspheres.

It has been reported in literatures that doxorubicin, epirubicin, rifampin and other drugs are prepared into microspheres with PLA and PGLA as the carrier materials, and they have good slow-release effect. The prepared PTX-PCL-MS has good slow-release effect in different release media. The results of PTX-PCL-MS release show the whole process of drug release from PTX-PCL-MS into the media, providing references for further study on in vivo release.

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