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Preparation, characterization and pharmacokinetics evaluation of Imperatorin lipid microsphere and its effect on proliferation of MDA-MB-231

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Abstract: Imperatorin is a chemical compound belong to Linear furan coumarins. Imperatorin is attracting considerable attention because of its anti-tumor, antibacterial, anti-inflammatory, anticoagulant and inhibition of myocardial hypetrophy and other pharmacological efficacy. However, imperatorin has limited water solubility and preferable lipid solubility, we decided to design and synthesize imperatorin lipid microsphere, to optimize preparation conditions. The aim was to develop and formulate imperatorin lipid microsphere through nano emulsion technology and apply the response surface-central composite design to optimize the imperatorin lipid microsphere formulation. Influence of content of amount of egg lecithin(A), amount of poloxamer188(B), soybean oil for injection accounted for the total percentage of oil phase(C) were investigated. Integrated effect of dependent variables including particle size(Y_1), polydispersity index (Y_2), Zeta potentials(Y_3), drug loading(Y_4), encapsulation efficiency(Y_5). Data of overall desirabiities were fitted to a second-order polynomial equation, through which three dimensional response surface graphs were described. Optimum experimental conditions were calculated by Design-Expert 8.06. Results indicated that the optimum preparation conditions were as follows: egg lecithin amount 1.39g, poloxamer188 amount 0.21g, soybean oil for injection amount 10.57%. Preparation of imperatorin lipid microsphere according to the optimum experimental conditions resulted in an overall desirability of 0.7286, while the particele size (168±0.54)nm, polydispersity index (PDI) (0.138±0.02), Zeta potentials (-43.5±0.5) mV, drug loading (0.833±0.27) $mg \cdot mL^{-1}$, encapsulation efficiency (90±1.27)%. The difference between observed and predicted values of the overall desirability of the optimum formulation was in range from 2.4% to 4.3%. Subsequently, using the Scanning electron microscopy to observe the micromorphology of imperatorin lipid microsphere, the result shows that round globular of relatively uniform and sizes within 200nm.The proliferation study of imperatorin lipid microsphere on MDA-MB-231 was investigated by MTT method. Furthermore, pharmacokinetics in Sprague Dawley rats were evaluated using orbital bleeding. A sensitive and reliable liquid chromatography with High Performance Liquid Chromatography (HPLC) method was established and validated for the quantification of imperatorin in rat plasma samples. The data were calculated by DAS

(Drug and statistics) pharmacokinetic software version3.2.6 (China). Results demonstrated that imperatorin lipid microsphere can significantly enhance the bioavailability of imperatorin and can significantly inhibit MDA-MB-231 cell proliferating. In conclusion, our results suggersted that the response surface-central composite design is suitable for the optimized lipid microspere formulation. Imperatorin Lipid microsphere can improve the bioavailability of imperatorin and inhibit the proliferation of MDA-MB-231 than that of imperatorin.

Key words: Imperatorin; Lipid microsphere; Response surface methodology; Pharmacokinetic

1.Introduction

Imperatorin is a chemical compound belong to linear furancoumarins and major extracted and isolated from the traditional herbal medicine of *Angelica dahurica*. Modern pharmaceutical studies have identified that imperatorin has good biological activity including analgesic, anti-bacterial, anti-inflammatory, vessel dilating and CYP450 inhibitory(1). Many research showed that imperatorin had certain anti-tumor efficacy such as inhibiting effect on human hepatocellular carcinoma cell line, human breast cancer cell line, human cervical carcinoma cell line, human osteosarcoma cell line and other tumor cell to metastasis. The mechanism mainly included the down regulation effect on MCl-1 protein expression mitochondrial(2,3,4,5). Joana Jakubowicz-Gil et al showed that imperatorin combined with quercetin can effectively inhibit the proliferation of tumor cells and induce the cell apoptosis(6,7,8). These suggested that imperatorin is a potential anti-cancer drug and have a good application prospect.

Imperatorin shows a relatively low bioavailability because of its poor water solubility. So it is difficult to prepare an ideal oral pharmaceutical preparation. It is also not easy to be developed to an injection. So its clinical application is limited and there is no clinical drug at present(9). Lipid microsphere injection is a kind of microsomal dispersion system with average particle size no more than 200nm, it is a monomolecular dispersing system with fatty oil as soft matrix and encapsulated by phospholipid membrane. As a new drug delivery system, lipid microsphere injection is an ideal injection carrier for lipid soluble drugs. Lipid microsphere is undoubtedly a suitable drug-loaded pattern for those small-molecule lipo-soluble drugs which have insolubility or poor solubility (10). In this study, we used the characteristics of poor solubility in water and good fat solubility, dissolves it in the injection of fat oil, and selected the lecithin as a emulsifier, combined with the nano emulsification technology to preparated the imperatorin lipid microsphere.

Uniform design and orthogonal design are two kinds of experimental design methods which are widely used in the research of pharmaceutical preparations of Chinese herbal medicine (11). But the uniform design and orthogonal design optimization method is constrained by linear model, it can only point out a direction of value factors but unable to find extreme, and the deviation between the measured value and the prediction is larger under the optimum preparation condition(12).

Response surface methodology (RSM) is a combination of mathematical and statistical techniques, which has the characteristics of fewer tests and higher test accuracy. It is also more simplified and comprehensive than orthogonal design and uniform design. In the process of optimization, practical research mainly focuses on central composite design (CCD) under RSM (13).Because CCD is very practically suitable for comparing experimental methodology with theoretical models(14), and it includes not only the effects of interation of the variables but also the overall effects of the parameters in the process(15), it is often used in the optimization method for the preparation of technology.

Breast cancer is one of the most common female cancers in the world. It is still associated with high morbidity and mortality. At present, chemotherapy and surgery are the important methods to treat breast cancer. Imperatorin is a chinese medicine monomer of traditional chinese medicine, which has the characteristics of high efficiency and low toxicity. Previous studies have showed that imperatorin has the anti-tumor effect(16,17), it has strong inhibition effect on MDA-MB-231(18). But because of its physical and chemical properties make its druggability is very low. In order to increase its druggability and exert its antitumor effect, optimization of preparation and formulation of lipid microsphere was accomplished, the pharmacokinetics of imperatorin in rats was investigated and the effect of imperatorin and imperatorin lipid microsphere on MDA-MB-231 proliferation was also compared in the study.

2 Materials and methods

2.1 Materials

2.1.1 Chemicals and Drugs

Imperatorin was purchased from the National Institutes for Food and Drug Control (batch: 110826 – 200511, Beijing, China). Soybean oil for injection (long chain triglyceride, LCT) and medium chain fatty acid glyceride for injection (MCT) were purchased from Tieling North Asia medicinal oil Co. Ltd (Liaoning, China). Egg lecithin was purchased from Dongshang biotechnology Co. Ltd (Shanghai, China). Glycerol for injection was purchased from Jiangxi Benefit Spectrum Health Pharmaceutical Division (Nanchang, China). Poloxamer188 was purchased from Shanghai Changsheng Technology Co. Ltd (Shanghai, China). The reagents were chromatography pure and analytical pure.

Fetal calf serum (FCS) and RPMI1640 were purchased from Hyclone (Thermo Fisher Scientific).Penicillin and Streptomycin solutions (10,000 U/mL Penicillin and 10,000 mg/mL Streptomycin) were purchased from Solarbio (Beijing Solarbio Science &Technology Co., Ltd., China). Non-essential amino acids were obtained from Sigma Chemical Co. (USA). Trypsin-EDTA solution (0.25% (w/w) trypsin/1 mM EDTA) was supplied by Gibco Laboratories (Life Technologies Inc., USA). MTT cell proliferation and Cytotoxicity Detection Kit (batch: 20170613, Jiangsu KeyGEN BioTECH Corp., Ltd).MDA-MB-231 cell were purchased from cell bank of Chinese Academy of Sciences.

2.1.2 Animals

Male Sprague-Dawley (SD) rats were purchased from Slack King Experimental Animal Center in Hunna (Hunan, China).Before the experiment, all rats were housed in an environmentally controlled room (25±2℃ and relative air humidity 52±20%), with free access to food and water. All animal experiments were approved by the Animal Center Committee of Jiangxi University of Traditional Chinese Medicine, all of which were conducted in full compliance with the local, national, ethical and regulatory princilpes.

2.2 Methods

2.2.1 Imperatorin lipid microsphere preparation

Imperatorin lipid microsphere was prepared with a high-speed shearing and high-pressure homogenization method as described previously(19,20). Imperatorin was dissolved in the oil phase, which composed of egg yolk lecithin, LCT and MCT. Water phase was composed of Glycerol, Sodium oleate and Poloxamer 188. The oil phase and water phase were all heated to the same temperature 70℃ respectively, and then the hot oil phase was added to the water phase and stirred in a high-speed shearing homogenizer for 10 min at a revolution speed of 19000rpm to obtain the colostrum. After then the colostrum was circulated 6 times with 600bar in the homogenizer. Imperatorin lipid microsphere was obtained.

2.2.2 Measurement of Size, PDI and Zeta Potential of Imperatorin lipid microsphere

The average particle size, PDI and zeta potential of lipid microsphere were measured using a Malvern laser particle size analyzer (Mal-vern, UK).Samples were diluted appropriately with double steamed water for the measurements, and zeta potential measurements were detected at 25 ℃.

2.2.3 Scanning electron microscopy (SEM)

The morphologies of the imperatorin lipid microsphere and blank lipid microsphere were observed using FEIQuanta 250 SEM (FEI Corporation, US). After dilution with double steamed water, drop on the sample stand, drain naturally and spraying for observation.

2.2.4 Determination of drug loading and encapsulation efficiency

Encapsulation efficiency was determined by an ultra-high speed centrifugation. In addition, the drug loading and encapsulation efficiency of imperatorin was determined following the solubilization of carriers in methanol and analysed by a high performance liquid chromatography (HPLC) method. The mobile phase consisted of methanol and double distilled water (80:20, v/v), A volume of 20μL of sample was injected and the flow rate was 1mL/min. The column temperature was maintained at 25℃, and the detection wavelength was set at 330nm(21).

The drug loading was calculated according to the standard curve.

Encapsulation efficiency (%) (22)= $(C_o V_o - C_w V_w)/ C_o V_o \times 100\%$

Drug loading=$(C_a W_b)/W_a$

2.2.5 RSM design and optimization of Imperatorin lipid microsphere preparation conditions

RSM was developed to acquire the optimal preparation conditions by establishing

the relationships between the variables and the response.

Based on the single factor test results of preliminary experiments and our previous studies, three formulation parameters, the amount of egg lecithin (A), amount of poloxamer188 (B), soybean oil for injection accounted for the total percentage of oil phase (C), were identified as key factors responsible for the particle size (Y_1), polydispersity index (Y_2), Zeta potentials (Y_3), drug loading (Y_4) and encapsulation efficiency (Y_5). The range and levels of the three independent variables used in this study and is summarized in Table1. The central composite design experiments were carried out in a randomized order, which included six repeated experiments to eliminate the system error. Dependent variables or responses were transformed into desirabiities mathematically by Hassan's method. Overall desirability was calculated from the geometric mean of five desirabilities of each formulation. In this method, we set the best value as 1, the worst value as 0, all desirabilities will be normalized from 0 to 1.

The formula to calculate the overall desirability was expressed as follows(23):

$$OD=(d_1d_2d_3d_4d_5)^{1/5}$$

$$d_{min}=(Y_{max}-Y_i)/(Y_{max}-Y_{min})$$

$$d_{max}=(Y_i-Y_{min})/(Y_{max}-Y_{min})$$

Where d is the overall desirability of each independent variable, d_1 d_2 d_3 d_4 d_5 is the overall desirability of particle size, particle size distribution, Zeta potentials, drug loading and encapsulation efficiency, respectively. Y is the determination value of each independent variable (i=1,2,3,4.5); Y_{max} and Y_{min} the maximum and minimum of each independent variables in all the tests.

Software of Design-expert 8.0 was used to analyze the experimental data of overall desirabilities and perform multiple regressions to obtain the coeffients of the cubic polynomial model, and to get the three dimensional response surface graphs. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was determined by F-test.

Table 1 Levels and code of variables chosen for central composite design.

Factors	code	Range and levels				
		-1.732	-1	0	1	1.732
egg lecithin	A	1	1.11	1.25	1.39	1.5
poloxamer188	B	0.1	0.21	0.35	0.49	0.6
Soybean oil /oil phase	C	0	10.57	25.00	39.43	50

2.2.6 Pharmacokinetics and Statistical Analysis

Six male Sprague-Dawley rats were given imperatorin lipid microsphere ($1mg\cdot mL^{-1}$) i.v. at a dose of 5mg/kg. Orbital blood sample (500μL) were collected at 2min,5min,10min,15min,20min,30min,45min,60min,90min,120min,180min,240min, 360min, after administration. Blood samples were placed in heparinized tubes, immediately centrifuged in a centrifuge tube coated with sodium heparin at $4000rmp\cdot min^{-1}$ at 4℃ for 10min. The supernatant were taken and stored at -80℃ until analysis. Plasma samples were treated by liquid-liquid extraction method. An HPLC method was developed for the determine of imperatorin. Pharmacokinetic parameters of imperatorin after intravenous injection of imperatorin lipid microsphere

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were calculated by DAS software. The mobile phase consisted of methanol and double distilled water (80:20, v/v), A volume of 20μL of sample was injected and the flow rate was 1mL/min. The column temperature was maintained at 25℃, and the detection wavelength was set at 330nm(21).

2.2.7 Effect of imperatorin and Imperatorin lipid microsphere on MDA-MB-231 proliferation

MDA-MB-231 cells were cultured in medium containing RPMI1640 (10% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, 100U/mL penicillin-streptomycin). The cells were maintained at 37 ℃ in an atmosphere containing 5% CO2 at 95% relative humidity. The medium was changed every other day during cell growth and differentiation. The cells could be used in the experiments when they had grown to 80%-90%. The cells were seeded onto 96-well plates at a density of 5×10^4, and discarded supernatant after grown for 24 h. Different concentration of imperatorin and Imperatorin lipid microsphere were added in and were cultured for different time (24、48、72 h). According to the instructions of MTT cell proliferation and cytotoxicity test kit to study the effect of imperatorin and Imperatorin lipid microsphere on MDA-MB-231 proliferating.

$$\text{Inhibition of cell proliferation (\%)} = \frac{\text{Control group OD} - \text{Drug group OD}}{\text{Control group OD}} \times 100\%$$

2.3 Data analysis

All experimental datas in this experiment were expressed as the mean ± standard error. Data analyses were performed by using the DAS 3.2.6 pharmacokinetic program (Chinese Pharmacology Society). All statistical analyses were analyzed using t-test.

3 Results and discussion

3.1 Central Composite Design of response surface methodology

The experimental data are summarized in Table 2. The statistical significance of the regression model was analyzed by P-value and F-test , and the analysis of variance (ANOVA) for the response surface quadratic model is shown in Table 3. In which the p-value<0.01 implied the model was very significant, p-value <0.05 suggested model term was significant. The p-value for the “Lack of Fit” test was 1.78, indicating the quadratic model was adequate.

By statistically processed and fitting, multiple regression equations were obtained as follows:

Final equation in terms of coded factors:

$OD=+0.51+0.082A-0.081B-0.011C-0.27AB-9.205E-003AC+9.205E-003BC-0.096A^2-0.097B^2-0.035C^2$

Final equation in terms of actual factors:

$OD=+0.50837+0.081881A-0.081132B-0.010991C-0.27387AB-9.20457E-003AC+9.20457E-003BC-0.095557A^2-0.096566B^2-0.035043C^2$

The analysis of fitting is shown in Table 4.

The above regression equations quantitatively described the relationship between the three independent variables (A, B, C) on index and the overall desirability. The

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adjusted R^2 for the predictive model of is 0.8918, and the statistical test results of equation parameters is summarized in Table 4. It is revealed that the experimental results adequately fitted the selected regression equations. The "Adj R-Squared" of 0.7944 is not as close to the "Pred R-Squared" of 0.4031 as one might mormally expect. This may indicate a possible problem or a large block effect with the model and/or data. Things to consider are response transformation, model reduction, outliers, etc. "Adeq Precision" measures the signal to noise ratio and a ratio greater than 4 is desirable. The ratio of 9.418 indicates an adequate signal. This model can be used to navigate the design space. It can be used predictively the obtain response value of a random formula within the range and level of independent factors by regression equations.

The better comprehend the predictive three-dimendional graphs of the models in the results, the response surface diagrams of imperatorin lipid microspere are shown in Figure1. The optimum formulation conditions were as follows: the amount of egg lecithin is 1.39g, the amount of poloxamer188 is 0.21g, and the amount of soybean oil for injection is 10.57g.

Using the recommended optimum conditions to test The suitability of the model equation for predicting the optimum response values. According to the model equation, using the RSM optimization approach to deterimined the optimum conditions.Three batches of imperatorin lipid microsphere were prepared according to the optimized formulation. Table 5 listed the optimum and their experimental and predicted values for the response variable under the test conditions, and the calculated percentage prediction error. Seen from Table 6, the prediction error of the response variables was found to vary between 2.4% and 4.3%. The results of verifying experiments were very close to the predicted values obtained from optimization analysis using desirability function with low prediction error, suggesting that the optimization was reasonable and reliable.

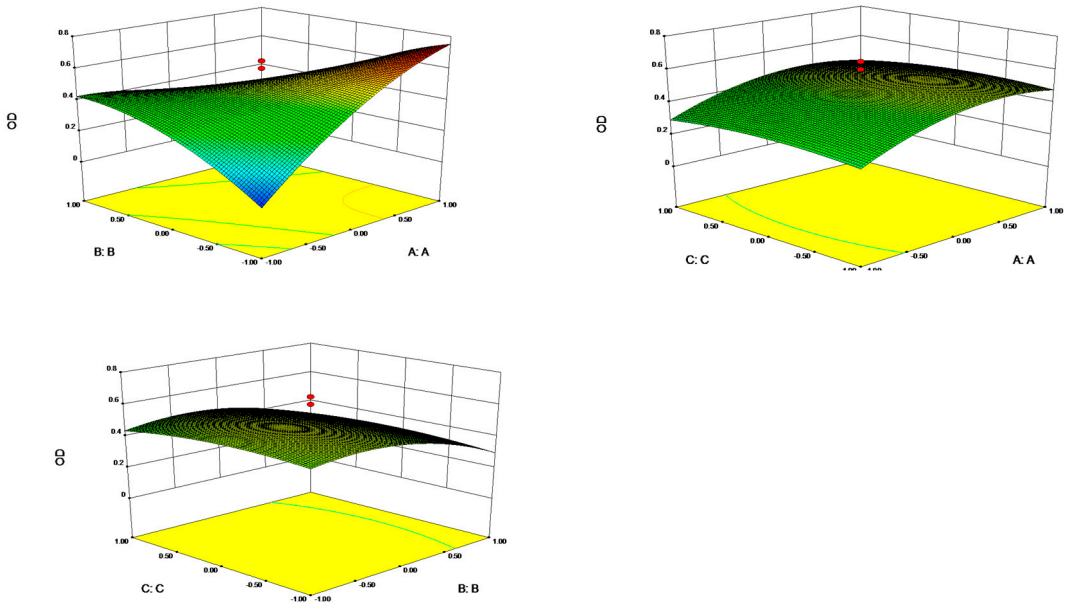

Figure1 Response surface plot

285 **Table 2 Variables and observed responses in central composite design for lipid microsphere.**

NO.	Levels of independent factors			Responses					
	A	B	C	Y_1	Y_2	Y_3	Y_4	Y_5	OD
1	1.11	0.21	39.43	177	0.148	-43.4	6.58	89%	0
2	1.25	0.35	25.00	172	0.131	-44.1	7.59	90%	0.4835
3	1.11	0.49	10.57	169	0.168	-47.0	8.27	89%	0.5195
4	1.25	0.35	25.00	172	0.128	-43.7	7.72	88%	0.4582
5	1.25	0.35	25.00	161	0.138	-45.0	7.29	89%	0.4562
6	1.39	0.49	39.43	164	0.097	-38.7	6.93	81%	0
7	1.25	0.35	25.00	165	0.148	-43.5	7.23	90%	0.4013
8	1.25	0.35	0	167	0.129	--43.4	7.16	84%	0.3693
9	1.11	0.21	10.57	193	0.122	-45.2	9.43	91%	0
10	1.0	0.35	25.00	201	0.132	-43.9	9.02	89%	0
11	1.5	0.35	25.00	168	0.134	-43.8	7.33	88%	0.4629
12	1.39	0.49	39.43	177	0.183	-41.9	9.14	89%	0.5391
13	1.39	0.49	10.57	154	0.116	-44.5	8.28	81%	0
14	1.11	0.49	39.43	165	0.094	-42.4	7.29	88%	0.4037
15	1.39	0.21	10.57	168	0.138	-43.5	8.33	90%	0.7286
16	1.25	0.35	25.00	164	0.120	-43.5	7.76	89%	0.6020
17	1.25	0.35	50.00	176	0.136	-44.7	7.88	90%	0.4567
18	1.25	0.35	25.00	170	0.129	-43.1	10.42	90%	0.6491
19	1.25	0.1	25.00	196	0.097	-41.5	10.92	92%	0.4569
20	1.25	0.6	25.00	170	0.096	-40.5	9.27	93%	0

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287 **Table 3 Statistical analysis of variance for the experimental results**

Source	Sum of squares	df	Mean square	F value	p-value prob>7
Model	1.05	9	0.12	9.16	0.0009significant
A-A	0.094	1	0.094	7.36	0.0218
B-B	0.092	1	0.092	7.23	0.0228
C-C	1.691E-003	1	1.691E-003	0.13	0.7233
AB	0.60	1	0.60	108.14	<0.0001
AC	6.778E-004	1	6.778E-004	0.053	0.8223
BC	6.778E-004	1	6.778E-004	0.053	0.8223
A^2	0.14	1	0.14	11.28	0.0073
B^2	0.15	1	0.15	11.52	0.0068
C^2	0.019	1	0.019	1.52	0.2462
Residual	0.13	10	0.013		
Lack of Fit	0.082	5	0.016	1.78	0.2713not significant
Pure Error	0.046	5	9.174E-003		
Cor Total	1.18	19			

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289 **Table 4 The results of fitting second-order equations**

Std.Dev.	0.11	R-Squared	0.8918
Mean	0.35	Adj R-Squared	0.7944

C.V. %	32.32	Pred R-Square	0.4031
PRESS	0.70	Adeq Precisior	9.418

Table 5 Constraints of factors and responses for optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Important
A:egg lecithin	is in range	1.0	1.5	1	1	3
B: poloxamer188	is in range	0.1	0.6	1	1	3
C:LCT/oil phase ratio	is in range	0	50	1	1	3
Responses: OD	maximize	0	0.7286	1	1	3

Table 6 The experimental and values for response (OD) along with percentage prediction error observed for the optimum test condition

Batch	A	B	C	OD		
				Predicted value	Experimental Value	Percent prediction error
20171101	1.39	0.21	10.57	0.7580	0.7286	3.8%
20171102	1.39	0.21	10.57	0.7580	0.7395	2.4%
20171103	1.39	0.21	10.57	0.7580	0.7251	4.3%

3.2 Drug loading and encapsulation efficiency

The drug loading and encapsulation efficiency of three batches was seen in Table 7.

Table 7 Drug loading, encapsulation efficiency of Imperatorin lipid microsphere ($\bar{x} \pm s$, n=3)

Batch	Drug loading (mg/ml)	encapsulation efficiency（%）
20171101	0.815	90.3
20171102	0.836	91.2
20171103	0.859	88.7
Mean	0.833±0.027	90.0±1.27

3.3 Particle size and Zeta potential measurements

The result of particle size and potential was shown in table8 and figure 2. From the results we can see that imperatorin lipid microsphere has the trait of small size and narrow size distribution.

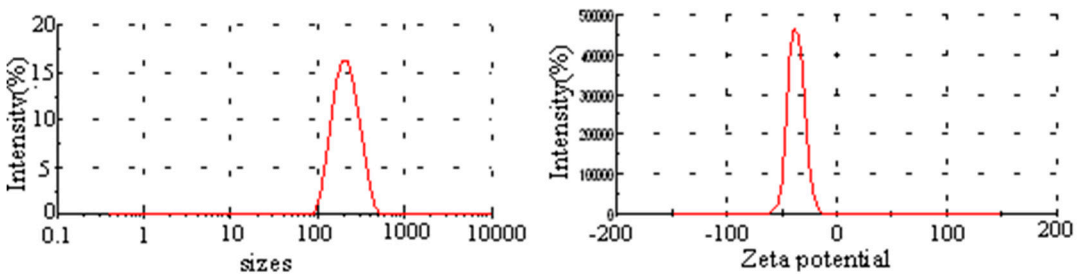

Figure 2 particle sizes and Zeta potential of Imperatorin lipid microsphere

Table 8 Zeta potential, particle size of Imperatorin lipid microsphere ($\bar{x} \pm s$, n=3)

Batch	Zeta potential (mv)	particle size（nm）	PDI
20171101	-43.1	169	0.114
20171102	-44.1	165	0.159
20171103	-43.5	169	0.142
Mean	-43.5±0.50	168±1.73	0.138±0.02

3.4 Scanning electron microscopy (SEM)

The SEM of Imperatorin lipid microsphere was shown in Figure 3. The imperatorin lipid microsphere was small homogenous vesicles with bilayer lipid membrane.

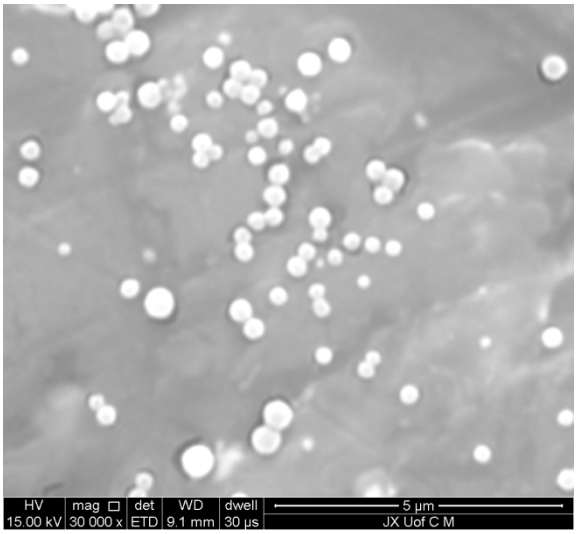

Figure 3. Scanning electron microscopy of Imperatorin lipid microsphere

3.5 Pharmacokinetic study

Because of poor solubility of imperatorin, it was not selected to compare with imperatorin lipid microsphere in the pharmacokinetic study. A dose of 5mg•kg^{-1} of imperatorin lipid microsphere was injected intravenously injection in rat. The major pharmacokinetic parameters were estimated using non-compartmental calculations performed with DAS (Durg and statistics) software version3.2.6 (China). The mean plasma concentration-time curves are shown in Figure 4. The major pharmacokinetic parameters are listed in Table 9.

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Upon IV administration at a dose of 5mg·kg^{-1}, the time to peak (maximum) concentration (T_{max}) was at 2 min after intravenous administration in rats, the peak (maximum) plasma concentration (C_{max}) of imperatorin lipid microsphere was 77.46± 23.82 mg·L^{-1}, indicating that imperatorin lipid microsphere could be quickly detected in plasma. Imperatorin lipid microsphere was shown to have a short half-life time (t1/2=0.998 ±0.396 h) and a clearance of 0.041±0.012 L·h^{-1}·kg^{-1}). The short half-life reminds us imperatorin lipid microsphere should be quickly metabolized in vivo. It should have a short duration of efficacy. The result suggested that we should carry some study on prolonging half-life of imperatorin lipid microsphere.

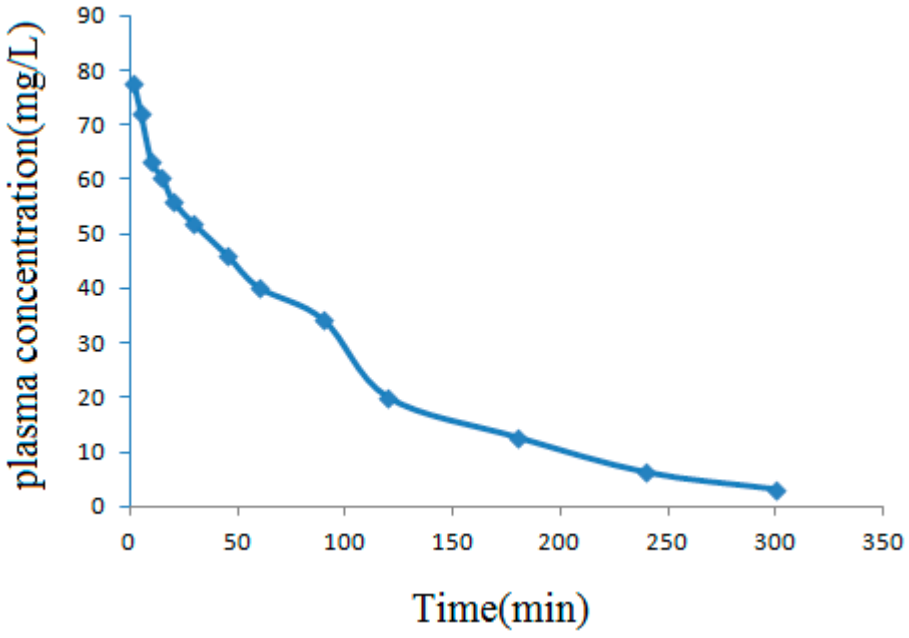

Figure 4. Mean plasma concentration-time curves after intravenous administration of 5mg/kg imperatorin lipid microsphere in rats.

Table 9The main pharmacokinetic parameters after intravenous administration of 5 mg·kg^{-1} imperatorin lipid microsphere in rats (mean ±SD, n=6)

Parameter	unit	intravenous injection
AUC(0-t)	mg/L*h	116.712±38.723
AUC(0-∞)	mg/L*h	121.244±40.012
AUMC(0-t)	h*h*mg/L	160.74±60.779
AUMC(0-∞)	h*h*mg/L	189.922±70.585
MRT(0-t)	h	1.377±0.412
MRT(0-∞)	h	1.566±0.512
VRT(0-t)	h^2	1.34±0.502
VRT(0-∞)	h^2	2.29±0.783
t1/2z	h	0.998±0.396
Tmax	h	0.0333±0.0106
Vz/F	L/kg	0.059±0.019
CLz/F	L/h/kg	0.041±0.012
Cmax	mg/L	77.46±23.82

3.6 Effect of imperatorin and imperatorin lipid microsphere on MDA-MB-231 proliferating

The results showed that the inhibition of imperatorin and imperatorin lipid microsphere on MDA-MB-231 proliferating all had positive correlation with the culture time (Figure 5). With the increasing of concentration of imperatorin or imperatorin lipid microsphere the inhibition rate of them on MDA-MB-231 proliferation improved correspondingly. Compared with the effect of imperatorin, imperatorin lipid microsphere group had stronger inhibitive effect on MDA-MB-231 proliferating than that of imperatorin.

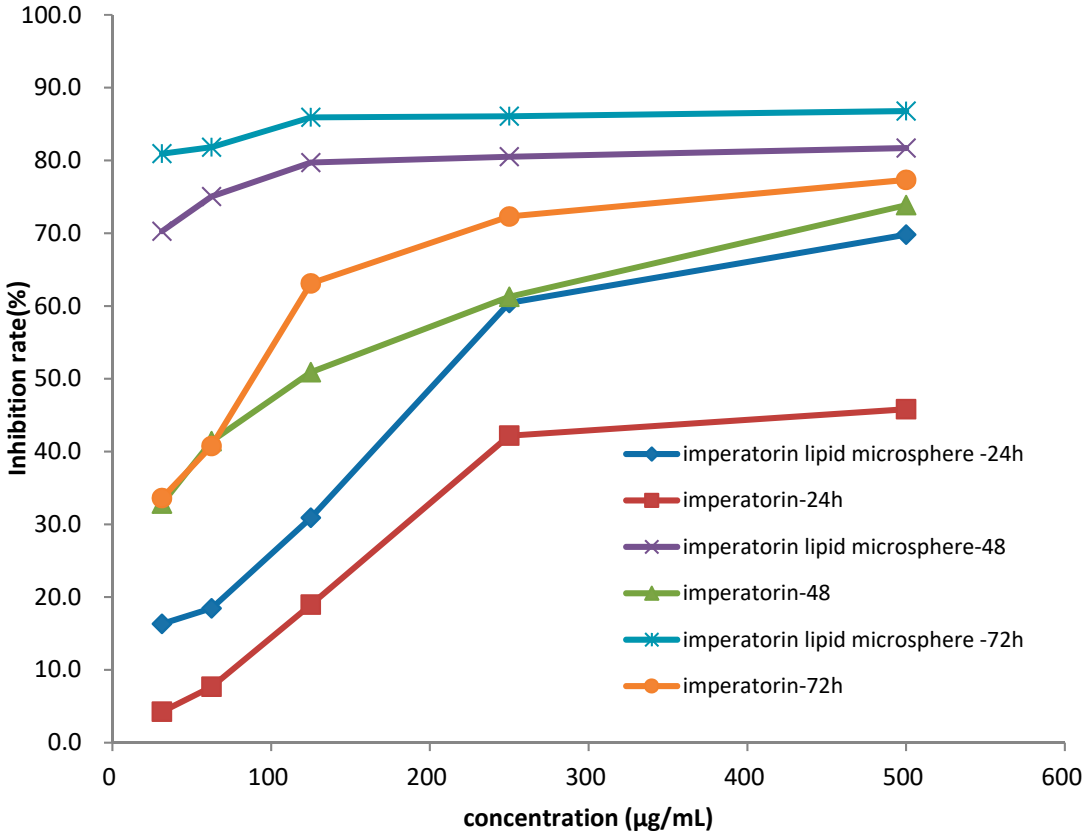

Figure 5 Inhibition cures of imperatorin and imperatorin lipid microsphere against MDA-MB-231

4 Conclusion

Lipid microsphere is a good candidate for drug loading because of its safety, stability, good biocompatibility, especially for those drugs with low solubility.

Central Composite Design-Response Surface Method is an optimal design method used in optimization of formulation due to its relatively small number of experiments and high precision. According to the surface change, three-dimensional effect of surface chart could directly response the influence of factors on the survey index. Based on the overlying of better condition chosen by multiple effects, the range of better conditions can be further reduced.

The optimum formulation was: egg lecithin 13.9g, poloxamer188 2.1g, and the

soybean oil 105.7g. The particele size was (168±1.73) nm, polydispersity index (PDI) was (0.138±0.02), Zeta potential was (-43.5±0.5) mV, drug loading (0.833±0.027) mg/ml, and the encapsulation efficiency was (90±1.27) %.The result showed that imperator lipid microsphere could significantly inhibit MDA-MB-231 cell proliferating. But pharmacokinetic study of imperator lipid microsphere showed that the half-time of imperator was very short. So it should be further study on how to increase its half-time and improve the residence time in blood.

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References

[1]Koziol E, Skalicka-Wozniak K. Imperatorin-pharmacological meaning and analytical clues: profound investigation. Phytochemistry Reviews. 2016; 15(4)：627-649.

[2]Choochuay K, Chunhacha P, Pongrakhananon V, Luechapudiporn R, Chanvorachote P. Imperatorin sensitizes anoikis and inhibits anchorage-independent growth of lung cancer cells. Journal of Natural Medicines. 2013; 67(3)：599-606.

[3]Luo KW, Sun JG, Chan JY, Yang L, Wu SH, Fung KP, et al. Anticancer Effects of Imperatorin Isolated from Angelica dahurica: Induction of Apoptosis in HepG2 Cells through both Death-Receptor- and Mitochondria-Mediated Pathways. Chemotherapy. 2011;57(6): 449-459.

[4] Huang ZP, Shao LL, Ruan YP. Anti-tumor Effect and Mechanism of Imperatorin Enhances the Cytotoxicity of Cisplatin Osteosarcoma Cells. Chin J Mod Appl Pharm. 2015; 32(10): 1193-1197.

[5]Zheng Y, Jiang K. Antitumor effect of imperatorin enhances cytotoxicity of doxorubicin to HeLa cells. Chinese Journal of Pathophysiology. 2015; 31(9): 1578-1583.

[6]Bqdziul D, Jakubowicz-Gil J, Paduch R, Glowniak K, Gawron A. Combined treatment with quercetin and imperatorin as a potent strategy for killing HeLa and Hep-2 cells. Molecular and Cellular Biochemistry. 2014; 392(12): 213-227.

[7] Jakubowicz-Gi J, Paduch R, Ulz Z, Badziul D, Glowniak K, Gawron A. Cell death in HeLa cells upon imperatorin and cisplatin treatment Cell death in HeLa cells upon imperatorin and cisplatin treatment. Folia Histochemica Et Cytochemica. 2012; 50(3): 381-391.

[8] Badziula D, Jakubowicz-Gila J, Lanqner E, Rzeski W, Glowniak K, Gawron A. The effect of quercetin and imperatorin on programmed cell death induction in T98G cells in vitro. Pharmacological Reports. 2014; 62(2): 292-300.

[9] Pirnay S, Bouchonnet S, Herve F, Libong D, Milan N, Ricordel I, et al. Develpoment and

validation of a gas chromatography-mass spectrometry method for the determination of imperatorin in rat plasma and tissue: application to study its pharmazokinetics. Journal of Chromatography B. 2009; 25(7): 869-873.

[10]Han F, Wei XX, Zhou MN, Zeng L, Shu JC, Yang M, et al. Research progress in traditional Chinese medicine injectable emulsion.Chinese journal of new drugs. 2015; 24(17): 1980-1984.

[11] Auriemma G, Mencherini T, Russo P, et al. Prilling for the development of multi-particulate colon durg delivery systems: pectin vs pectin-alginate beads. Carbohydr Polym. 2013; 92(1): 367-373.

[12] Watts PJ, Illum L. Colonic durg delivery. Drug Dev Ind pharm. 1997; 23(9): 893-913.

[13] Rose F, Wern JE, Inqvarsson PT, van de Weert M, Andersen P,Follmann F, et al. Engineering of a novel adjuvant based on lipid-polymer hybrid nanoparticles: a quality-by-design approach. J. Control. Release. 2015; 210: 48–57.

[14] Xu HT, Paxton J, Lim J, Li Y, Wu ZM. Development of a gradient high performance liquid chromatography assay for simultaneous analysis of hydrophilic gemcitabine and lipophilic curcumin using a central composite design and its application in liposome development. J. Pharm. Biomed. 2014; 98: 371–378.

[15] Varshosaz J, Ghaffari S, Khoshayand MR. Development and optimization of solidlipid nanoparticles of amikacin by central composite design. J. Liposome Res. 2010; 20: 97–104.

[16] Wang M, Chen JW, Li X. Study on antitumor activity of five Furanocoumarins from the root bark of Changium smyrnioides in vitro. Chinese Journal of Experimental Traditional Medical Formulae. 2012; 18(6): 203-205.

[17]Chen H. Apoptosis of human breast cancer cell line MCF-7 induced by orbixin. Zhejiang Practical Medicine June. 2015; 20(3): 177-179.

[18]Yang XW, Xu B, Ran FX, Wang RQ, Wu J, Cui JR. Inhibitory effect of 40 Coumarins Compounds against growth of human epidermal carcinoma a cell line A432 and human mammary cancer cell line BCAP in vitro. Mod Chin Med. 2006; 8(12): 9-10.

[19] WANG Yue-qi, TANG Xing, CAI Cui-fang. Study of tacrolimus-loaded lipid microsphere preparation [J]. Chinese Journal of Pharmaceutics.2014,12(5):167-176

[20] LIU Ai-na, CHEN Hao, TANG Xing. Preparation and physical stability of astragaloside IV lipid microspheres for injection. Chinese Journal of Pharmaceutics.2009; 7(4):290-298.

[21] Chinese pharmacopoeia [S]. 2015.

[22]Yang SY, Chen JY, Zhao D, Han D, Chen XJ. Comparative study on preparative methods of DC-Chol/DOPE liposomes and formulation optimization by determining encapsulation efficiency. Int. J. Pharm. 2012; 434: 155–160.

[23]Wu W, Cui GH, Lu B. Optimization of multiple evariables:application of central composite design and overall desirability. Chin Pharm J. 2000; 35(8): 530-533.