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Study on the Preparation of Doxorubicin Hydrochloride Liposomes and Their Therapeutic Effect on Liver Cancer

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Abstract: *Objective*: This study aimed to prepare doxorubicin hydrochloride liposomes and explore their application value in patients with liver cancer. *Methods*: Doxorubicin hydrochloride liposomes were prepared using the ammonium sulfate gradient method. Doxorubicin, as a broad-spectrum antitumor drug, has significant toxic and side effects after toxicological investigation. After preparing DOX-Lip, single-factor analysis was used to analyze the effects of solution pH, number of ultrafiltration, oil-water ratio, incubation temperature, and time on the encapsulation efficiency of doxorubicin hydrochloride liposomes. The process was optimized through orthogonal experiments and then applied clinically. 110 patients with liver cancer were selected as the research subjects to verify the drug's effectiveness. *Results*: The results of this study showed that under optimal process conditions, the prepared doxorubicin hydrochloride liposomes were evenly distributed, similar to spherical shapes, with an average particle size of 85–87 mm and a Zeta potential of 15–16 mV, indicating good encapsulation efficiency. The application of these liposomes to clinical treatment of liver cancer demonstrated good therapeutic effects and could effectively promote favorable patient prognosis. *Conclusion*: The doxorubicin hydrochloride liposomes prepared through process optimization exhibit strong stability and pronounced sustained-release characteristics, providing a solid foundation for the treatment of liver cancer.

Keywords: Doxorubicin hydrochloride; Liposomes; Drug preparation; Liver cancer; Clinical efficacy

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1. Introduction

Liver cancer is a common malignant tumor in clinical practice, with high morbidity and mortality rates, mainly dominated by hepatocellular carcinoma [1]. There are no obvious clinical symptoms in the early stages of the disease, but in the middle and late stages, patients may experience pronounced right upper quadrant abdominal pain and aversion to greasy foods [2]. Some patients may also experience weight loss and lower extremity edema,

which seriously threatens their health. Currently, the main therapeutic drugs for hepatocellular carcinoma include fluorouracil, etc. [3], which can inhibit the development of tumor cells but also have certain side effects. Based on this, this study prepared doxorubicin hydrochloride liposomes using the ammonium sulfate gradient method, achieving an encapsulation efficiency exceeding 95%, which effectively solved the problem of low encapsulation efficiency. Furthermore, patients with liver cancer who visited our hospital from October 2023 to October 2024 were selected as research subjects to explore the application effects of doxorubicin hydrochloride liposomes in the treatment of liver cancer, as detailed below.

2. Materials and methods

2.1. Reagents and instruments

Reagents: Doxorubicin hydrochloride (Ouyi Pharmaceutical Co., Ltd. of Shijiazhuang Pharmaceutical Group), hydrogenated soy phosphatidylcholine (Hubei Wei's Chemical Reagent Co., Ltd.), pegylated phosphatidylethanolamine (Jiangsu Southeast Nano Materials Co., Ltd.), cholesterol (Anhui Kebao Bioengineering Co., Ltd.), ammonium sulfate (Shandong Jinruida Biochemical Co., Ltd.), histidine (Zhengzhou Yuhe Food Additive Co., Ltd.), sucrose (Zhengzhou Dewang Chemical Industry Co., Ltd.), ethanol (Anhui Shuanghui Biological Industry Co., Ltd.), isopropanol (Shandong Xuchen Chemical Technology Co., Ltd.), ammonium chloride (Shandong Lubei Chemical Co., Ltd.), sodium chloride (Shouguang Chenlong Chemical Co., Ltd.).

Instruments: Particle size analyzer (Beijing Yaou Depeng Technology Co., Ltd.), electronic balance (Kunshan Lugong Precision Instrument Co., Ltd.), constant temperature heating magnetic stirrer (Zhengzhou Shiji Shuangke Experimental Instrument Co., Ltd.), constant temperature water bath shaking tank (Changzhou Gaode Instrument Manufacturing Co., Ltd.), cryo-transmission electron microscope (Shuimu Keyi Technology Co., Ltd.).

2.2. DOX Measurement method

2.2.1. Chromatographic conditions

The specific chromatographic conditions are set as follows: Chromatographic column: XDB-C18 (4.6 × 250 mm); Mobile phase: 0.28% sodium dodecyl sulfate solution - methanol - acetonitrile; Wavelength: 254 nm; Flow rate: 1.0mL·min^{-1}; Column temperature: 30 ℃.

2.2.2. Specificity test

Select the control solution, and compete for the drug-loaded liposomes and blank liposomes, dilute with methanol, then take the supernatant, and analyze it according to the chromatographic conditions to investigate its specificity. Subsequently, the linear relationship was investigated. Precision measurement involves the vector configuration of DOX reference substances to form a standard storage solution with a concentration of 0.2 mg·ml^{-1}. Then, a certain amount of solution was taken to prepare solutions with concentrations of 14, 16, 20, 24, and 26 μg·mL^{-1}, respectively, and analyzed according to the corresponding conditions, and the corresponding standard curve was drawn.

2.2.3. Preparation of doxorubicin hydrochloride liposomes

Prepare doxorubicin hydrochloride liposomes by the ammonium sulfate gradient method. Dissolve lipid components such as hydrogenated soy phosphatidylcholine, cholesterol, and pegylated phosphatidylethanolamine

in organic solvents to form a lipid solution [4]. Use a rotary evaporator or thin-film dispersion method to remove organic solvents and form a lipid film. Prepare an ammonium sulfate solution and use it to hydrate the lipid film to obtain a suspension containing blank liposomes. Use a high-pressure homogenizer or extruder to prepare the blank liposome suspension into liposomes with uniform particle size. Use tangential flow technology or column chromatography to remove ammonium sulfate outside the liposomes, forming an ammonium sulfate gradient inside and outside the phospholipid membrane [5]. Dissolve doxorubicin hydrochloride in an appropriate solvent to prepare a drug solution. Under heating conditions, mix the drug solution with blank liposomes to allow the drug to diffuse into the liposomes driven by the ammonium sulfate concentration gradient. Post-processing: Use methods such as dialysis or ultrafiltration to remove unencapsulated drugs and impurities. Add excipients such as sugar and buffers to adjust the stability and pH of the liposomes. Sterilize by filtration, and dispense to obtain the finished product [6].

2.2.4. Liposome characterization

For particle size distribution, take the corresponding amount of doxorubicin hydrochloride liposome solution, place it in the sample cell, and measure the Zeta potential using a particle size analyzer. For morphological determination, observe the morphology of liposomes using an electron microscope. Select a certain amount of doxorubicin hydrochloride liposomes, place them on a copper grid, and observe their morphology after freezing. For encapsulation efficiency testing, use a glucose gel filtration method to detect doxorubicin hydrochloride liposomes. The calculation methods are shown in formulas 1 and 2.

$$\text{Encapsulation Efficiency} = \left([DOX]_{\text{Encapsulated}} / [DOX]_{\text{Total}} \right) \times 100\% \qquad \text{(Formula 1)}$$

$$\text{Recovery Rate} = \left([DOX]_{\text{Encapsulated}} + [DOX]_{\text{Free}} \right) / [DOX]_{\text{Total}} \times 100\% \qquad \text{(Formula 2)}$$

2.2.5. Single factor analysis method

Using DOX encapsulation efficiency as the observation index, factor analysis was performed on the pH of the ammonium sulfate solution, oil-water ratio, extrusion times, ultrafiltration times, incubation temperature, and incubation time.

2.3. Statistical methods

The statistical data involved in this study were processed and calculated using SPSS 21.00 software. Chi-square test was selected for measurement data, and *t*-test was used for counting data. When the calculation result shows $P < 0.05$, it means that the difference is statistically significant.

3. Results

3.1. Establishment of content determination method

In the specificity test, detection was carried out according to chromatographic conditions. Under such chromatographic conditions, the reference solution and the drug-loaded liposomes reached the maximum absorption peak at 254 nm, and the blank liposomes did not show a chromatographic peak at the same retention time, indicating high specificity. See **Figure 1** for details.

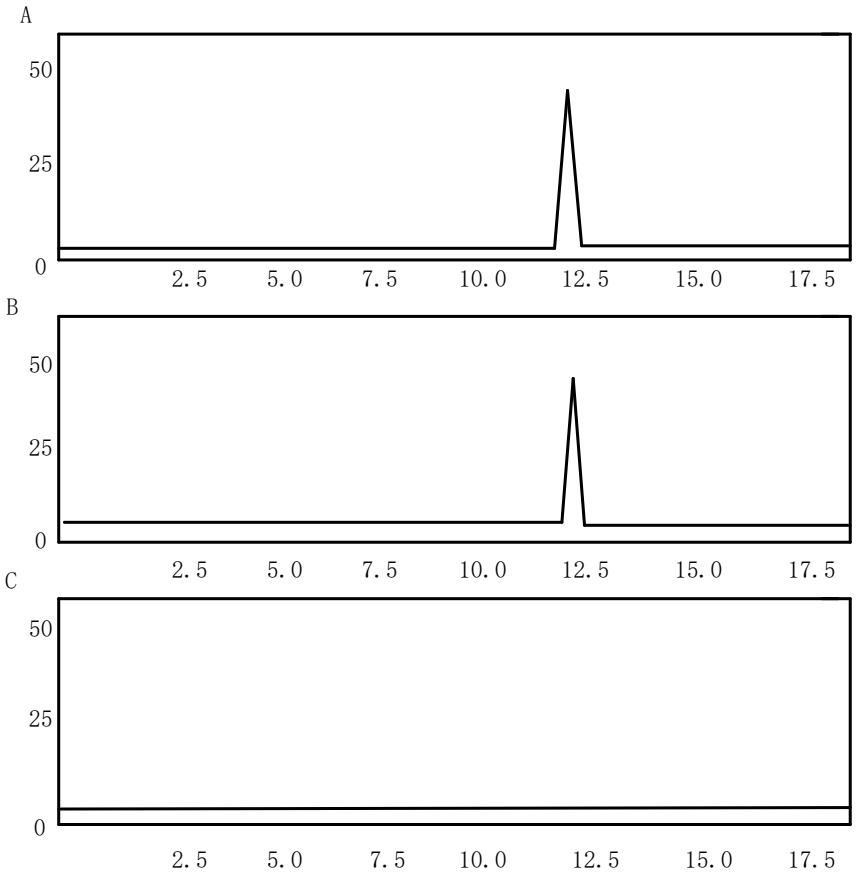

Figure 1. Results of specific chromatogram.

3.2. Recovery test

The configured solution was analyzed by chromatographic conditions, and the study results found that the recovery rates were all above 95%, meeting the HPLC content requirements.

3.3. Single factor analysis

Through single-factor analysis, it was found that ammonium sulfate solution is one of the main influencing factors of liposome encapsulation efficiency. When the pH value increases, there is no significant change trend in the encapsulation efficiency of liposomes. As the number of ultrafiltrations increases, the encapsulation efficiency of liposomes mainly shows a trend of increasing first and then decreasing. When the oil-water ratio is 1:6–1:10, as the oil-water ratio decreases, but the volume of ethanol is small, the concentration of the filter paper solution increases, and the particle size also increases. Therefore, the oil-water ratio is set to 1:10. As the number of extrusions increases, the particle size decreases. After 8-10 extrusions, there are no significant changes in sample particle size and encapsulation efficiency. Therefore, the number of extrusions is set to 10. Through orthogonal experiments, the preparation process of optimized liposomes was discovered. See **Table 1** for details.

Table 1. Analysis of orthogonal experiment results

Trial	Factor A	Factor B	Factor C	Factor D	Encapsulation efficiency
1	1	1	1	1	85.62
2	1	2	2	2	91.33
3	1	3	2	3	97.46
4	2	1	2	3	80.64
5	2	2	3	1	93.75
6	2	3	1	2	91.52
7	3	1	3	2	73.21
8	3	2	1	3	82.27
9	3	3	2	1	90.89
K1	274.25	239.50	259.43	270.28	-
K2	265.98	267.33	262.88	256.07	-
K3	246.39	279.88	264.41	260.38	-
k1	91.50	79.80	86.48	90.10	-
k2	88.65	89.14	87.60	85.36	-
k3	82.14	93.30	88.15	86.80	-
R	9.36	13.49	1.68	4.74	-

3.4. Analysis of application effects

Compared with the control group, the total effective rate of treatment in the observation group was significantly higher, and the difference was statistically significant ($P < 0.05$). See **Table 2** for details.

Table 2. Comparison of clinical efficacy results between the two groups

Group	Cases	Markedly effective, *n*(%)	Effective, *n*(%)	Ineffective, *n*(%)	Total effective rate, n(%)
Control group	55	18 (32.73)	27 (49.09)	10 (18.18)	45 (81.82)
Observation group	55	21 (38.18)	32 (58.18)	2 (3.64)	53 (96.36)
χ^2	-	-	-	-	5.986
P	-	-	-	-	0.014

4. Discussion

Doxorubicin hydrochloride, also known as Adriamycin hydrochloride, has a relatively broad antibacterial spectrum. However, during the treatment process, doxorubicin hydrochloride also exhibits certain drug resistance [7], limiting its clinical application. Relevant studies have directly pointed out that doxorubicin hydrochloride liposomes, as a nano-drug preparation, can be prepared by the ammonium sulfate gradient method with an encapsulation efficiency exceeding 95% [8]. Moreover, doxorubicin hydrochloride liposomes have a certain inhibitory effect on RNA and DNA synthesis and play a significant role in liver cancer cells. Due to the influence of liposomes, they can not only prolong the half-life of the drug but also reduce its side effects, thereby controlling

the disease [9]. As a novel drug carrier, liposomes have shown great potential in the field of drug delivery since their discovery in the 1970s, owing to their unique biocompatibility, degradability, and targeting ability. Liposomes are composed of phospholipid bilayers that can encapsulate hydrophilic or hydrophobic drugs and deliver them precisely to tumor tissues through passive or active targeting [10]. Additionally, liposomes can extend the circulation time of drugs in the bloodstream, reduce exposure to non-target tissues, thereby reducing toxicity and improving efficacy.

Based on this, the present study prepared doxorubicin hydrochloride liposomes using the ammonium sulfate gradient method and applied them in clinical settings. The results indicated that the final determined process involved an oil-water ratio of 1:10, an extrusion time of 8, a fusion pH of 5 for ammonium sulfate, a temperature of 55 ℃, and an incubation time of 20 minutes. The prepared doxorubicin hydrochloride liposomes exhibited good processability. Clinical validation revealed that they could effectively improve clinical efficacy.

5. Conclusion

In summary, doxorubicin hydrochloride liposomes can be prepared using the ammonium sulfate gradient method and applied in clinical settings to enhance clinical efficacy. However, further clarification of the correlation between drug release in vitro and in vivo is needed to ensure the consistency between doxorubicin hydrochloride liposomes and RLD.

Disclosure statement

The authors declare no conflict of interest.

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