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Preparation of Nanoparticles of Digoxin Solid Lipid

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In order to prove the role of solid lipid nanoparticles in oral absorption and bioavailability, the biodegradable monoglyceride with low toxicity and good biocompatibility is used as lipid materials to prepare nanoparticles of digoxin solid lipid and carry out research on polyethylene glycol aimed at the low utilization of digoxin in this research, in order to provide experimental basis for the development of new preparation system of digoxin. The results of the research show that the solid lipid nanoparticles prepared by the combination of ionic compound and solvent diffusion method can achieve effective encapsulation and high-efficiency load of water-soluble digoxin, and improve the absorption effect and bioavailability. Based on the preparation of polyethylene glycol-modified nanoparticles of digoxin solid lipid, it is expected to reduce the amount of usage and side effects of digoxin.

1. Introduction

Solid lipid nanoparticle (SLN) is a new type of nanocarrier that has become a hot topic in recent years. It is made of natural or synthetic lipid materials such as glycerin triacetate, compound glyceride and waxes with a particle size between 50 and 1000 nm. SLN is of high stability, less leakage, high bioavailability, low toxicity and other characteristics, suitable for industrial production (Aghaei-Amirkhizi et al. , 2016). At present, solid lipid nanoparticle is considered to be an ideal preparation, which can be distributed in a large area in the body and has good physical stability (Batool et al., 2014). The concentration of the solid lipid nanoparticle can be kept stable for a long time and has a release and regulation effect. In recent years, with the deepening of research on SLN, a large number of literatures have reported that SLN can improve bioavailability. Studies have reported that organisms encapsulated by solid lipid nanoparticles are of high availability for the cyclosporine A with low utilization, and the encapsulated half-life solution in vivo can be extended several times (Cao et al., 2014).
The digoxin materials are used to prepare digoxin compound by ion complex method, and then the nanoparticles of digoxin solid lipid are prepared by solvent diffusion method, which provides experimental and theoretical basis for the development of new use system of digoxin (Chen et al., 2017).

2. Research on synthesis and physical and chemical properties of nanoparticles of digoxin solid lipid

The digoxin materials are used to prepare digoxin-phospholipid complex by ion complex method. The structure of the complex is confirmed by differential scanning calorimetry (DSC). Monoglyceride is used as lipid material to prepare nanoparticles of digoxin solid lipid by solvent diffusion method. The particle size and shape, encapsulation efficiency and in vitro release and other physical and chemical properties of digoxin solid lipid nanoparticles with different modification ratio of polyethylene glycol are studied through adjustment and control of the dosage of polyethylene glycol 2000-stearate (PEGzooo-SA) (0.5%, 10%).
The size and distribution of solid lipid nanoparticles are measured by particle size analyzer; the shape of solid lipid nanoparticles is observed by transmission electron microscopy; the digoxin content is determined by HPLC method; the entrapment efficiency and loading amount are determined by centrifugal filtration and the in vitro release characteristics of solid lipid nanoparticles are inspected by dialysis bag (Chen et al., 2017).

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2.1 Preparation of digoxin- phospholipid complex

The digoxin-phospholipid complex is prepared by ion complex method. The method comprises the following steps: accurately weighing lecithin (1.5 mg) and digoxin (10 mg) in an eggplant-shaped flask; adding appropriate amount of ethanol (2mL) to dissolve the mixture, and steeping for 20 minutes at 50□ to form digoxin-phospholipid complex in the shape of uniform film.

2.2 Structure verification of digoxin- phospholipid complex

A differential scanning calorimetry (DSC) curve is drawn by taking four samples of lecithin, digoxin material, physical mixture of lecithin and digoxin material and compound of lecithin and digoxin. Determination method: empty aluminum crucible is used as reference compound, and the other crucible is used to place samples. Scanning speed is 10□/min; scanning range is 0-150 □. DSC curve is recorded (How et al., 2013).

2.3 Preparation of digoxin solid lipid nanoparticles

The digoxin solid lipid nanoparticles are prepared by solvent diffusion method. The specific method is as follows: taking digoxin-phospholipid complex film; add 1mL of absolute ethanol to elute the film as organic phase, and then respectively adding accurate amount of glycerin monostearate and PEG2000-SA into the organic phase and heating in a water bath at 50 °C to completely melt the lipid material; rapidly injecting it into 9 ml of water bath with thermostatically distilled water of 50□ at a stirring speed of 400 rpm and continuously stirring for 5 minutes; cooling to room temperature to obtain nanoparticle solution of digoxin solid lipid. The solid lipid nanoparticles with PECT2000-SA mass ratios of 0%, 5% and 10% in prescription are written as SLN, pSLN-5% and pSLN-10% respectively (Lin et al., 2017). Table 1 shows the dosage prescription table of monoglyceride and PEG:

Table 1: The composition of lipid materials for preparation of SLN and pSLN

Prescription	Glyceryl monostearate(mg)	PEG2000-SA(mg)
1	25	0(0%)
2	22.5	2.5(5%)
3	20	5(10%)

3. Results and discussion

3.1 Verification of the structure formed by digoxin phospholipid complex

The differential scanning calorimetry (DSC) is used to verify the formation of digoxin-phospholipid complex. DSC is a thermal analysis method that measures the energy difference between a sample and reference compound with temperature variation under the condition of temperature programming. In differential scanning calorimetry, the relationship between the heat applied to maintain the temperature difference between a sample and reference compound per unit time and temperature is a DSC curve (Ma et al., 2012). The vertical axis of the curve presents the heat applied per unit time and the horizontal axis temperature or time. The area of the curve is proportional to the change of enthalpy and can be used to study the composition of compound, change of nucleic acid and membrane structure. In order to verify whether the complex of digoxin and lecithin is formed, four samples of lecithin, digoxin material, physical mixture of lecithin and digoxin material as well as compound of lecithin and digoxin are taken to draw the differential scanning calorimetry (DSC) curve (Mansour et al., 2016).

It can be seen from Figure 1 that the DSC curve is significantly different from the physical mixture of digoxin and lecithin after combination of digoxin and lecithin by ion complex method, of which the endothermic peak at 120°C disappears, and the rest peak area of the endothermic peak is changed. The phase transition temperature is reduced to 70□ and the peak shape is significantly increased compared with the phase transition peak of physical mixture of digoxin and lecithin. The results show that when the mass ratio of lecithin and digoxin is 1.5:1, the structure is changed after reaction by ion complex method, and the ionic bond is produced to form the ion complex of digoxin and lecithin (Wang et al., 2015).

3.2 Size and morphology of digoxin solid lipid nanoparticles

The particle size and distribution coefficient (PI) of digoxin solid lipid nanoparticles prepared are measured by dynamic light scattering (particle size and surface potential meter), and the size and morphology of digoxin solid lipid nanoparticles are observed by transmission electron microscopy. The results are shown in Table 2. It can be seen from the table that the particle size of SLN without PEG modification is about 275 nm, and the particle size of pSLN prepared decreases with the increase of PEG modification. When the PEG modification

quality is 10%, the particle size is about 158 nm. This is because the hydration of PEG on the surface of solid lipid nanoparticles is enhanced after modification by hydrophilic PEG, and the three-dimensional stability of the nanoparticles is improved. Therefore, the particle size of the prepared nanoparticles is reduced, which is consistent with the light transmission of nanoparticle dispersion liquid.

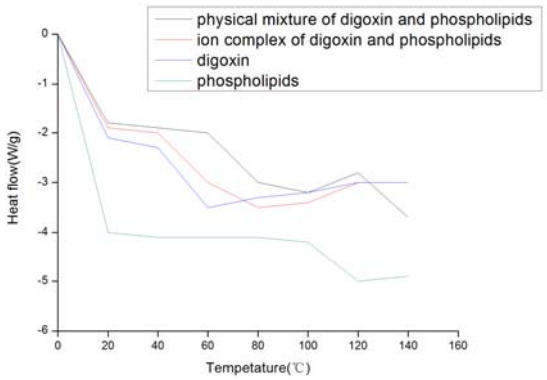

Figure 1: DSC curves of digoxin, phospholipids, digoxin phospholipid complex and physical mixture of lecithin

Table 2: The size and its polydispersity index (PI) of prepared SLN and pSLN. (n=3)

PEG-SA content (W t%)	Size (nm)	PI
0	275±34.90	0.509
5	220±22.45	0.443
10	158±15.95	0.449

3.3 Entrapment efficiency (EE) and load (DL) of digoxin solid lipid nanoparticles

The content of digoxin is determined by HPLC, and the standard curve of digoxin aqueous solution is established. The standard curve is obtained by regression of digoxin concentration (C, μg/mL) and peak area: y = 62.191x-34.85, r2 = 0.9963. There is a good linear relationship between peak area and concentration in the concentration range of 1-20 μg/mL for digoxin (Table 3 and Figure 2).

Table 3: The calibration curve of digoxin solution

Concentration(μg/mL)	1	2	5	10	15	20
Peak area	44.8	95.7	259.2	592.7	851.1	1244.5
Result calculation	y=62.191 x -34.685 r^2=0.9963					

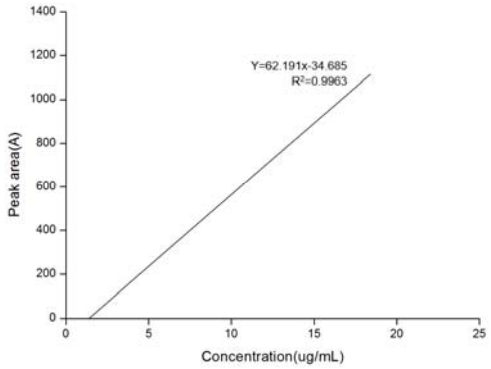

Figure 2: Linear relationship between HPLC peak area and digoxin solution and concentration

Similarly, the standard curve of digoxin can also be determined according to the HPLC method. The standard curve is obtained by regression of digoxin concentration (C, μg/mL) and peak area: y = 54.676x -1.1022, r2=0.9992, that is, there is a good linear relationship between peak area and concentration for digoxin in the concentration range of 0.02-10 μg/mL, as shown in Figure 3:

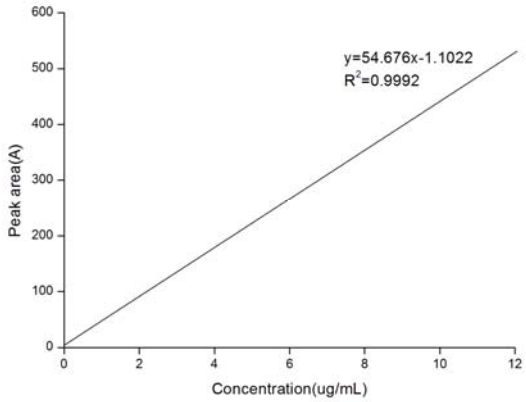

Figure 3: Linear relationship between peak area obtained from HPLC and digoxin solution.

The standard curve of digoxin in HBSS can be obtained by regression of digoxin concentration (C, μg/mL) and peak area: y=66.325x+1.4916, r2=0.9988 (0.05-2 μg/mL), that is, there is a good linear relationship between concentration and peak area for digoxin in HBSS in the concentration range of 0.05-2 μg/mL. the specific simulation analysis is shown in Figure 4:

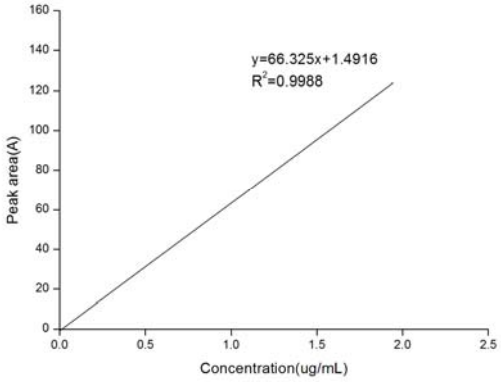

Figure 4: Standard curve of digoxin solution in HBSS

3.4 Research on in vitro release of digoxin solid lipid nanoparticles

The in vitro release behavior of digoxin solid lipid nanoparticles and the impact of different proportions of PEG modification on in vitro release behavior are studied by dialysis bag method, taking PBS of pH7.4 as release medium and digoxin solution as a control. The release curve is shown in Figure 5. It can be seen from the figure that the diffusion rate of digoxin solution is relatively slow through the membrane of the dialysis bag, indicating that the diffusion of the dialysis bag membrane has a certain retardation effect. Compared with the diffusion of digoxin solution through the dialysis bag membrane, the release of digoxin solid lipid nanoparticles is significantly slowed down, indicating that solid lipid nanoparticles have a sustained release effect on digoxin, and its 72-hour cumulative release is 48.67%. Release shows two-phase release behavior, namely initial burst release and sustained slow release thereafter. The results show that with the increase of PEG modification ratio, the difference of solid lipid nanoparticles is mainly reflected in the initial burst stage.

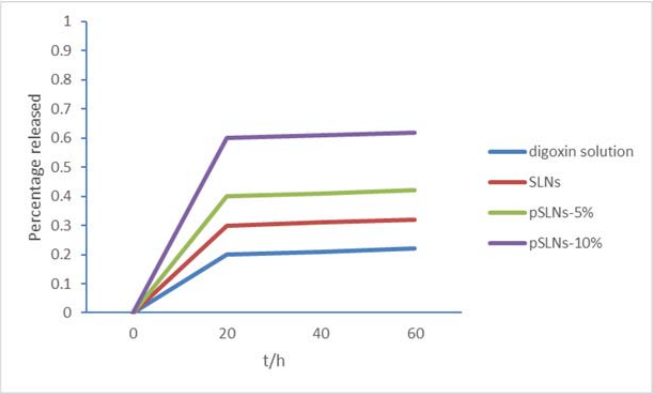

Figure 5: In vitro digoxin release profiles from SLN and pSLN in phosphate buffered saline (pH 7.4) (n=3)

3.5 Cytotoxicity evaluation of digoxin solid lipid nanoparticles

The principle of MTT assay for inspection of cytotoxicity is as follows: The exogenous dye generates water-insoluble purple crystalline formazan under the reduction of succinate dehydrogenase in the mitochondria of living cells, and the production of the latter is related to the number of living cells. Formazan can be dissolved with DMSO. The absorbance can be determined at a wavelength of 570nm with enzyme-linked immunoassay, which can indirectly reflect the cell growth and proliferation activity. The relationship between cell survival and concentration of SLN and pSLN is shown in Figure 6.

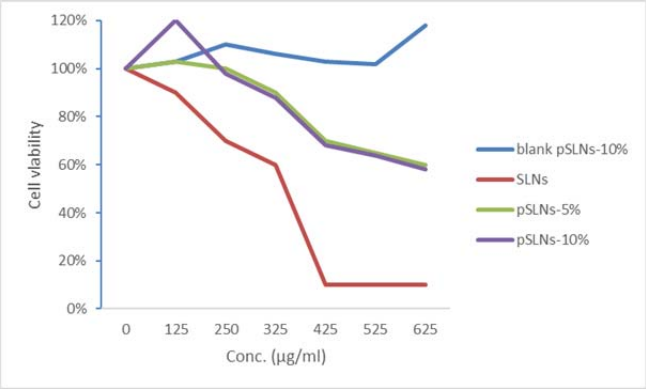

Figure 6: Cytotoxic effect of SLN and pSLN incubated with MDCK cells (n=3)

It can be seen from Figure 6 that the median lethal dose (IC50) of SLN on MDCK cells is 269.0 ug/mL, while the IC50 of pSLN-5% and pSLN-10% is 549.25 and 550.47 ug/mL. It can be seen that the cytotoxicity of nanoparticles decreases with the increase of PEG modification ratio. The results of the simulation show that SLN and pSLN have lower cytotoxicity. When the nanoparticle concentration is 100 ug/mL, the cell survival rate is close to 100%, and the cell activity is affected. Therefore, 100 μg/mL of the nanoparticle concentration is used for transport and uptake in the follow-up experiments.

4. Conclusion

When the mass ratio of digoxin to lecithin is 1: 1.5, digoxin can effectively compound with lecithin to form a digoxin-lecithin ion complex. Therefore, the digoxin solid lipid nanoparticles prepared by solvent diffusion method can effectively encapsulate and efficiently load water-soluble digoxin with an encapsulation efficiency of 99.19% and loading capacity of 19.87%. The encapsulation efficiency decreases after modification by polyethylene glycol, but it is still above 95%. The diameter of digoxin solid lipid nanoparticles is about 275 nm, and the particle size decreases with the increase of PEG modification. When the PEG modification ratio is 10%, the particle size is reduced to about 158 nm; the in vitro release result shows that the digoxin solid lipid nanoparticles have a sustained release effect with the 72-hour cumulative release of 48.67%, and its release rate slows as the increase of modification proportion of polyethylene glycol.

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