

Preparation and Characterization of Pulmonary Surfactant-Super Oxide Dismutase Liposomes

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Citation

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Abstract

Objective: To prepare pulmonary surfactant-superoxide dismutase (PS-SOD) Liposomes and analyze the biological characteristics of them.

Methods: To prepare PS-SOD Liposomes with rotary evaporation method and analyze the formulation and bioactivity of them, including shape, size, encapsulating ratio, antioxidant activity and surface tension.

Results: The PS-SOD Liposome is a kind of ivory white suspension and presents to be a fairly homogeneous spherical article with a mean grain size of $0.463 \pm 0.223 \mu\text{m}$. There is no change in antioxidant activity before and after SOD being encapsulated to be PS-SOD Liposomes. No difference has been found in surface tension between PS and PS-SOD Liposomes.

Conclusion: PS-SOD Liposomes can be successfully prepared through rotary evaporation method and maintain stable biological activities both of PS and SOD.

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INTRODUCTION

The pathological mechanism of the respiratory insufficiency after extracorporeal circulation is systemic inflammatory reaction and pneumonic ischemic reperfusion. Oxygen free radicals play a major role in this procedure^[1].

Super oxide dismutase (SOD) is proved to be a kind of effective mundificant of oxygen free radicals but it cannot be effectively transported into the lung tissues either through oral application or intravenous infusion^[2]. As a giant protein molecule, the half life period of SOD is short and it is not easy for SOD to enter the pneumocytes to bring into full play as the role of mundificant scavenging oxygen free radicals^[3,4].

Pulmonary surfactant (PS) is widely known as a kind of phospholipids mixture which can form liposomes with other molecules and has good compatibility with cell membrane. In further studies, we conceive to prepare PS -SOD liposomes to have more SOD transported into pneumocytes with PS and lead a joint action in protecting lung functions.

MATERIALS AND METHODS

THE PREPARATION OF PS-SOD LIPOSOMES

Dissolve 1 ml PS (Curosurf, 80mg·ml⁻¹, Chies Company, Italy) into 3 ml diethyl ether and 1 ml methanol; then, add 1 ml SOD solution (3x10⁶U·L⁻¹, Sigma company, USA) into the mixture. Use the ultrasound oscillator with a constant temperature bath to let the mixture become an emulsion, 10-15 mins. for the first time; then, again repeat after a 5 mins interval. Using rotary evaporation to remove the organic solvent from the mixture, the remnant is gelatum. Adding 2 ml buffer solution 0.01mol·L⁻¹, pH 7.4 into the gelatum and after being oscillated with a mixer, a kind of suspension is formed. This is the PS-SOD Liposome.

ANALYSIS OF THE FORMULATION AND BIOACTIVITY OF PS-SOD LIPOSOMES

We analyzed the shape, size, encapsulating ratio, anti-oxygen activity and surface tension of the PS-SOD Liposome.

SHAPE AND SIZE OF THE PS-SOD LIPOSOME

We took a look at the shape and size of the PS-SOD Liposome treated with negative staining under a transmission electron microscope. First of all, we dissolved PS-SOD liposomes in ammonium acetate (10mmol·L⁻¹) together with Tris buffer solution (50mmol·L⁻¹, pH7.5) to the concentration of 10mmol/l. Then, we dropped a drip of

liposome solution on a piece of membrane supported with a copper screen. Thereafter, we dried the liposome drip with filter paper and dropped several drips of ammonium molybdate solution in order to dissolve the dried liposome. We let it dry naturally for 30 minutes, then put the membrane under the transmission electron microscope and took a look at the shape of the liposome and measured its size. We selected ten pictures from the photos taken with different field of vision under the transmission electron microscope and measured the diameter \bar{d} of the liposome. The grain size(\bar{d}) of the liposome is the mean diameter multiplied by 0.707, which is the correction factor.

ENCAPSULATING RATIO OF PS-SOD LIPOSOMES

Dilute 0.5 ml PS-SOD liposomes suspension to 5 ml with phosphate buffered solution; then, centrifuge(1.0×10^6g , 4) for 30 minutes. Remove the supernatant liquid with SOD not encapsulated in it. Dilute the remnant containing PS-SOD liposomes bursula to 1 ml with phosphate buffered solution. Measure the protein in the PS-SOD liposome suspension and the PS-SOD liposome bursula with the same method, lowry method, respectively. We can then calculate the encapsulating ratio.

ANTIOXIDANT ACTIVITY OF PS-SOD LIPOSOMES

Dilute 10 μ l SOD solution to the concentration of 1mg/l with destillala aquae concentratac and measure the Antioxidant activity with SOD Assay Kit(Cayman Inc). Dilute 100 μ l PS-SOD liposomes to the concentration of 1mg/l with Triton100(100g·L⁻¹) and destillala aquae concentratac, then measure the Antioxidant Activity with SOD Assay Kit(Cayman Inc) and compare the antioxidant activity of PS-SOD liposomes with SOD solution.

SURFACE TENSION OF PS-SOD LIPOSOMES

Dilute 100 μ l PS to 200 μ l with phosphate buffered solution and measure the 5-minute-surface tension with a surface tension tensiometer. Then, measure the surface tension of the PS-SOD liposome with the same method and find the difference.

RESULTS

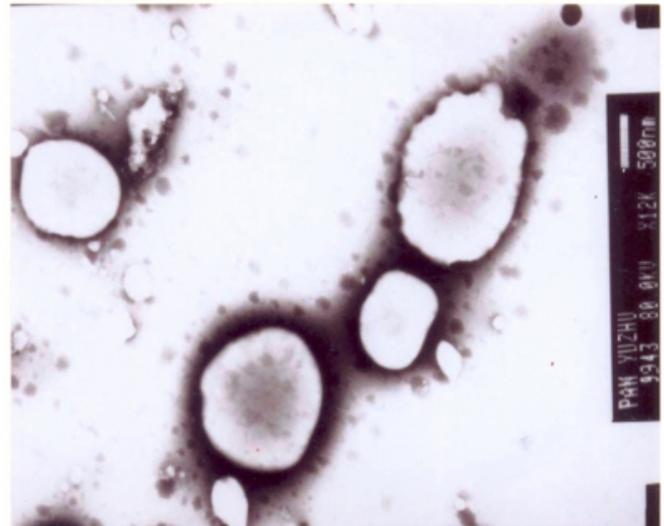
1) Morphous of the PS-SOD liposome

The PS-SOD liposome prepared with the method of rotary evaporation is a kind of ivory white suspension and presents to be fairly homogeneous spherical article under a transmission electron microscope. Every PS-SOD liposome

consists of ectal adipose membrane with high electron density and SOD solution with low electron density inside. (Pit 1.)

Figure 1

Figure 1: The PS-SOD liposome consists of ectal adipose membrane with high electron density and SOD solution with low electron density inside and presents to be fairly homogeneous spherical article under the transmission electron microscope. 80kvx9



2) The grain size(\bar{d}) of the PS-SOD liposome

The mean grain size of the PS-SOD liposome is $0.463 \pm 0.223 \mu\text{m}$ n30.

3) Encapsulating ratio of PS-SOD liposomes

While the contents of the protein in PS-SOD liposome suspension is 0.55g·L⁻¹, PS-SOD liposome bursula contains 0.22g·L⁻¹ protein. The encapsulating ratio is 40.

4) Antioxidant activity of the PS-SOD liposome

While absorbance value of SOD solution is 0.262 ± 0.011 n8), the absorbance value of PS-SOD liposome is 0.272 ± 0.013 n8. There is no difference whether the SOD is being encapsulated or not. ($t=1.721, P=0.2970.05$)

5) Surface tension of the PS-SOD liposome

While the surface tension of PS is 14.15 ± 0.60 mN/m n8), the surface tension of the PS-SOD liposome is 12.78 ± 0.54 mN/m n8). We cannot find any differences between them. ($t=4.811, P=0.7050.05$)

DISCUSSION

In 1965, Professor Bangham and Colleagues[5] found the

liposome. From then on, lots of researchers concentrated on the study of the use of liposomes as drug carrier, including formulation, grain size, stability, pharmacokinetic and pharmacodynamics of liposomes, and great improvement was achieved on how to prolong the residence time of liposomes in the body. Nowadays, the liposome is widely used in the delivery of anti-tumor and anti-infection drugs^[6,7].

The outer layer of the liposome is a bimolecular leaflet of lipid, which consists of adipoid and protein. Even though, all kinds of lipids can be used to prepare liposomes, phospholipids are commonly used. Pulmonary surfactants, a lipid-protein mixture secreted by type alveolar cell can also be used to prepare liposomes theoretically.

There are several other methods to prepare liposomes such as the film dispersion method, injection method, dialysis method and rotary evaporation method^[8]. We chose the rotary evaporation method in the preparation of liposomes because the rotary method is fit for the preparation of liposomes containing water-solubility drugs. Dissolve pulmonary surfactants in diethyl ether and add some methanol to make the solution more completely. Use ultrasound to make the mixture to be uniform monophase. Remove the organic solvent with reduction vaporization and dilute the remnant in the phosphate buffered solution. After being oscillated, we got liposomes.

There are three main biological characteristics of liposomes: Biocompatibility, the phospholipid used to prepare liposomes is moiety of cellular membrane and can be used as the carrier of drugs that cannot transit through the cellular membrane. Encapsulation, a kind of physical procedure, has no influence on the molecular structure of the drugs that can be encapsulated effectively; no matter what kind of the material it is, eg. water-solubility or liposolubility. On account of this, we prepared liposomes with pulmonary surfactants and superoxide dismutase assuming that superoxide dismutase encapsulated in liposomes can be transported through the cellular membrane into the cell effectively. Biological degradation, liposomes can be degraded by the specific lipase in endochylema. Delayed release and controlled release, because of the delayed release and controlled release effects, liposomes extend the action time of the superoxide dismutase^[9].

Observing and determining the liposome under the transmission electron microscope is one of the most common and easiest method as well as the negative staining

method is fit for the liposome whose grain size is less than 5 μm . There are two kinds of negative staining methods: the spraying method and the spotting method. We chose the last one. Since the diameter of the liposome determined from the pictures taken under the transmission electron microscope is the diameter of the flatted liposome, the real diameter of the liposome must be calculated by being multiplied by 0.707, the correction factor. The diameter of the liposome we prepared through rotary evaporation is $0.463 \pm 0.223 \mu\text{m}$, and is basically not different from most of the research reported before. Pulmonary surfactant-superoxide dismutase (PS-SOD) liposomes belong to big monolayer liposomes^[8].

It is reported that the encapsulating ratio of liposomes varied from 35%-65%, mainly related with variety, composition, purity of the phospholipid and ionic strength in the buffer solution^[10]. The encapsulating ratio of PS-SOD liposomes we prepared was 40%, and is relatively small possibly due to the pulmonary surfactant being a mixture of phospholipid and protein with a low purity and unreasonable constituent ratio.

Not only did ultrasound and temperature changes in the process of preparing liposomes with rotary evaporation easily lead to protein denaturation, but also the superoxide dismutase degenerating itself, which is the natural character of enzyme. It is necessary to detect the activity change of pulmonary surfactant and superoxide dismutase before and after the preparation. It has been proved in our study that there has been no difference detected according to the activity of superoxide dismutase and the surface tension between before and after the preparation. This means that the PS-SOD liposome has a stable biological activity. Due to the success of preparing PS-SOD liposomes, it is believed that liposomes can combine both the activity of pulmonary surfactant and superoxide dismutase together to educe synergistic effect of protecting lung functions during and after the extracorporeal circulation period.

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