

Study on Targeting and *in vitro* Anti-oxidation of Baicalin Solid Lipid Nanoparticles

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Abstract: **Objective** To prepare liver-targeted baicalin solid lipid nanoparticles (BSLNs) and to study their *in vitro* anti-oxidative activity. **Methods** BSLNs were prepared by emulsification ultrasonic dispersion method and characterized by transmission electron microscopy and laser particle size distribution; The tissue *in vivo* distribution was detected by pharmacokinetics; *In vitro* anti-superoxide dismutase (SOD) activity and reduction capacity of BSLNs were determined; The ability of removing hydroxyl radical was determined by phenanthroline-Fe²⁺ oxidation. **Results** The best prescription was baicalin-soybean lecithin-glyceryl monostearate-poloxamer 188 (1:5:15:30); The encapsulation efficiency and drug loading were 84.7% and 5.65%, respectively, mean size of particles was (68.6 ± 8) nm, Zeta potential was -22.13 mV; The *in vitro* anti-oxidant results showed that BSLNs had a significant inhibitory effect on SOD and a strong reducing capacity as well as a removing hydroxide radical ability. The targeting rate of BSLNs was 6.931 for liver. **Conclusion** The results demonstrate that BSLNs could enhance the liver targeting ability and *in vitro* anti-oxidative activity significantly.

Key words: baicalin; *in vitro* anti-oxidative activity; liver-targeted; solid lipid nanoparticles; tissue distribution

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Introduction

Baicalin was extracted from the roots of *Scutellaria baicalensis* Georgi (Labiateae) commonly used in the combination with other herbs in traditional Chinese medicines. Baicalin has a lot of pharmacological effects, such as anticancer, antibacterial, antiviral activities. It was used to treat the hepatitis B and liver fibrosis in clinic currently. The oral dosage forms of baicalin were widely used, but the efficiency was not satisfying. In order to improve the therapeutic effect of baicalin, baicalin solid lipid nanoparticles (BSLNs) were prepared (< 100 nm). Nano-level drug could be ingested by *in vivo* macrophage of liver tissue in which the drug was concentrated, therefore the liver tissue would be targeted. BSLNs could provide more better treatment against hepatitis B and liver fibrosis.

Materials and methods

Materials and reagents

Baicalin (purity > 95%) was purchased from Zhucheng Haotian Medicine Co., Ltd. (China) and detected by RP-HPLC. Glyceryl monostearate was obtained from Tianjin Bodi Chemical Industry Co., Ltd. (China). Soybean lecithin was obtained from Shanghai Taiwei Medicine Co., Ltd. (China). Poloxamer 188 was obtained from Shanghai Yunhong Chemical Industry Co., Ltd. (China). Superoxide dismutase (SOD) Assay Kit-WST was purchased from Dojindo Laboratories, Inc. (Kumamoto, Japan). Sprague-Dawley (SD) rats were from Dalian Medical University (China). All the chemical reagents used were of analytical grade.

Preparation of BSLNs

The preparation technology was optimized by the L₉(3⁴) orthogonal design. Based on the single factor

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experiment, four factors were selected: baicalin/soybean lecithin (A), soybean lecithin/glyceryl monos (B), baicalin/poloxamer 188 (C), and baicalin quality (D). The optimum formulation was baicalin-soybean lecithin-glyceryl monostearate-poloxamer 188 (1:5:15: 30), and poloxamer 188 was dissolved in distilled water and added into soybean lecithin solution. The preparation was treated by magnetic stirring at 72 °C to keep aqueous phase; Glycerol monostearate was treated by magnetic stirring at 72 °C until completely dissolved, then baicalin solution was added to constitute organic phase. The aqueous phase was dripped into organic phase, kept on stirring for 10 min at 72 °C, pulverized for 10 min, and filtered by 450 nm micropore membrane.

Particle size and Zeta potential

The mean size and Zeta potential of BSLNs were measured by Zeta PALS/90 plus, at 25 °C using samples diluted (1:2) appropriately with super distilled water.

Particle morphology

The morphology of BSLNs was observed by transmission electron microscope (TEM), Hitachi—500 Instrument. Before analysis, the samples were diluted (1:10) with distilled water, dropped in copper grids, and stained with 2% phosphotungstic acid.

Entrapment efficiency and drug loading efficiency

The entrapment efficiency (EE) and drug loading efficiency (DLE) were determined by HPLC method and the mobile phase was methanol-water-phosphoric acid (55:45:0.2) on C₁₈ column (250 mm × 4.6 mm, 5 μm). The analysis was carried out at a flow rate of 1 mL/min at room temperature with the detection wavelength of 279 nm.

The EE of BSLNs was determined by ultrafiltration centrifuge method. BSLNs solution (0.5 mL) was put into ultrafiltration centrifuge tube to centrifugate in 5000 r/min, at 2 °C for 10 min. The supernatant containing free drug was withdrawn by means of HPLC at the detection wavelength of 279 nm. The broken emulsion method was used to determine the total concentration of baicalin. The supernatant was determined by HPLC with the detection wavelength of 279 nm. Formulas are as follows:

$$EE = (AUC_{total} - AUC_{free}) / AUC_{total} \quad (1)$$

$$DLE = EE \times w_1 / w_2 \quad (2)$$

Where AUC_{free}: peak area of free drug, AUC_{total}: peak area of total drug, w₁: amount of baicalin quality, w₂: amount of lipid materials

In vivo distribution

Chromatographic condition The experiment was performed on an Agilent 1100 Series HPLC System, the analysis was carried out at room temperature on an analytical Agilent C₁₈ column (250 mm × 4.6 mm, 5 μm), and the mobile phase for HPLC analysis consisted of a mixture of methanol-water-phosphoric acid (55:45:0.2), which was filtered and degassed under reduced pressure prior to use. The analysis was carried out at a flow rate of 1 mL/min with the detection wavelength of 279 nm.

Preparation of standard curve The blank plasma or tissue homogenate was precisely measured, added into different concentration of baicalin-methanol solution and internal standard (IS), respectively.

Animals grouping and administration After an overnight fast, 48 SD rats were divided into two groups, BSLNs group and baicalin group, respectively by the tail-vein injection of BSLNs and baicalin solution at a dose of 30 mg/kg. The plasma samples (1 mL) from the two groups via the orbital were collected into 2.5 mL heparinized tubes in 0.25, 0.5, 1, 2, 4, 6, and 8 h after the administration, then immediately centrifuged to be separated, finally kept at -20 °C until analysis. Then the rats were sacrificed to take the tissues of heart, liver, spleen, lung, and kidney (Li and Yang, 2008) and the tissue samples were stored at -20 °C for analysis.

Sample treatment The plasma samples were mixed with IS (Carbamazepine) and acetonitrile. Samples were vortex-mixed for 1 min and centrifuged at 14 000 r/min for 10 min. The supernatants were dried by water bath at 55—60 °C (He and Li, 2008).

Each tissue (300 mg) was added into 1.5 mL sodium chloride solution to prepare the tissue homogenate. Tissue samples followed to the plasma treatment method.

In vitro anti-oxidative activity

Determination of SOD activity The BSLNs solution and baicalin solution were prepared with the concentration of 10—500 μg/mL, respectively. According

to the kit instructions, the samples were determined on Microplate Reader at 450 nm.

Reducing ability Fe^{3+} could be reduced to Fe^{2+} by reducing substances. The reaction between Fe^{2+} and phenanthroline could form prunosus complex compound (Weng, 1993; Hai, 2002). Baicalin solution and BSLNs solution with the concentration of 10–1000 $\mu\text{g/mL}$ were prepared, respectively. The control group is VC solution. All reagents were added into 96 hole culture plate. The sample hole was filled with 90 μL solvent A (2 mmol/L FeCl_3 -2 mmol/L phenanthroline, 1:1) and 10 μL sample solution; control hole with 90 μL solvent A and 10 μL VC solution; and blank hole with 100 μL distilled water. All of them reacted for 30 min at room temperature, then were determined by Microplate Reader at 516 nm.

Hydroxyl radical scavenging The capability of hydroxyl radical scavenging was investigated by the phenanthroline- Fe^{2+} oxidation method (Jin and Cai, 1996; Yu, Zhang, and Cao, 2007). The preparation of BSLNs solution and baicalin solution with the concentration of 10–1000 $\mu\text{g/mL}$ was the same as “Reducing ability”. The sample hole was filled with 90 μL solvent B (5 mmol/L phenanthroline-7.5 mmol/L FeSO_4 -2.5 mmol/L EDTA, 1:1:1), 10 μL sample solution, and 10 μL 0.1% H_2O_2 solution; control hole with 90 μL solvent B, 10 μL VC solution, and 10 μL 0.1% H_2O_2 solution; and blank hole with 100 μL solvents B. All of them reacted for 1 h at 37 $^\circ\text{C}$, then were determined by Microplate Reader at 536 nm.

Statistical analysis

All the data were expressed as $\bar{x} \pm s$. The significant differences between the two groups were statistically analyzed using Student's *t* test. The differences among the multiple groups were evaluated by a One-way analysis of variances (ANOVA), followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

Results

Characterization of BSLNs

Particle size and Zeta potential According to Fig. 1, the mean particle size of BSLNs was (68.6 ± 8) nm, Zeta potential indicated the negative charges on BSLNs surface was -22.13 mV, and the results showed that BSLNs solution system was relatively stable.

Particle morphology

As shown in Fig. 2, the appearance of BSLNs was regularly and similarly spherical.

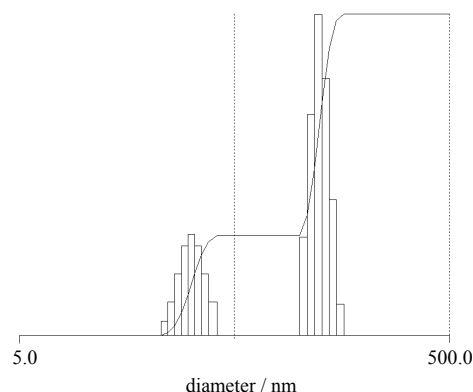


Fig. 1 Particle size distribution of BSLNs

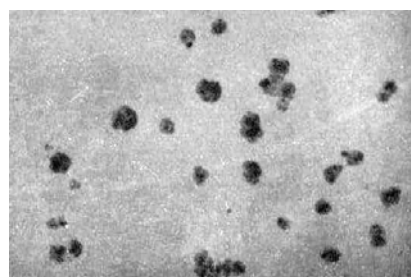


Fig. 2 TEM photograph of BSLNs

In vivo distribution

In contrast to the baicalin concentration in each tissue, the tissue distribution in the two groups was significantly different. It confirmed that the targeting of BSLNs to the liver was more obvious than that to the other tissues (Fig. 3).

Targeting evaluation

Drug targeting index (DTI) is used to compare two preparations on liver tropism difference and to calculate the DTI in each organ. The results were shown in Fig. 4. The formula is as follow.

$$\text{DTI} = \text{AUC}_p / \text{AUC}_s$$

Where p refers to BSLNs and s refers to baicalin

From Fig. 4, the DTI of BSLNs in the liver was significantly higher than that in the other tissues. The targeting of BSLNs to the liver is better than that of baicalin.

In vitro anti-oxidative activity

Determination of SOD activity SOD inhibitory rate of BSLNs was higher than that of baicalin in the range 10–500 $\mu\text{g/mL}$, the two groups had significant difference as shown in Fig. 5. The SOD inhibitory

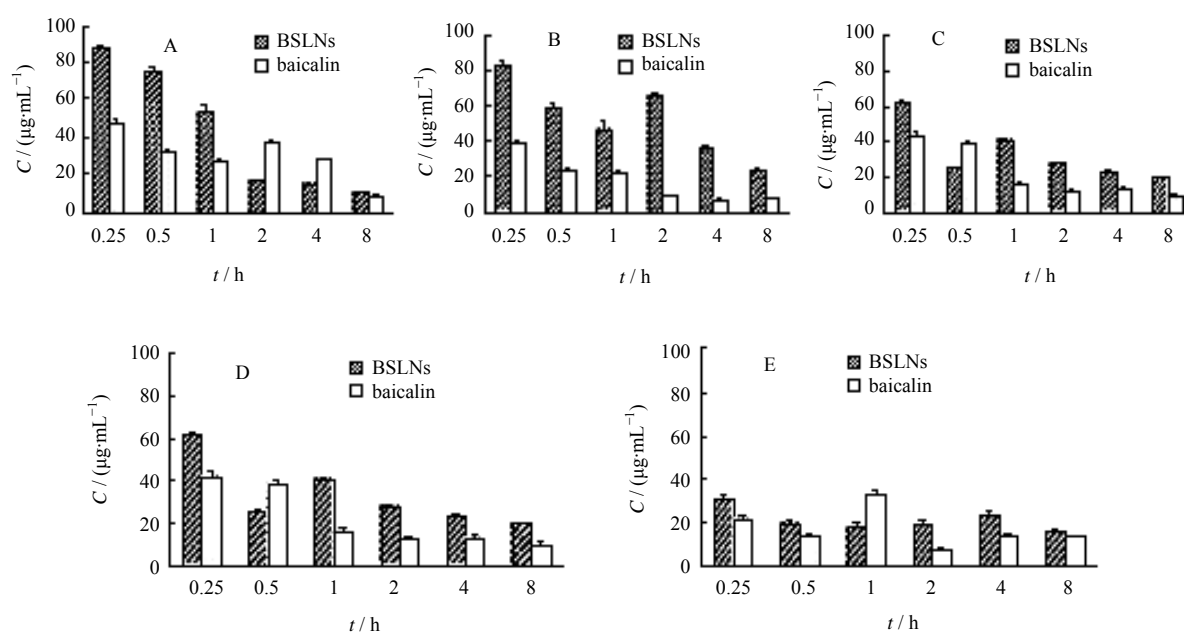


Fig. 3 Concentration of baicalin in heart (A), liver (B), spleen (C), lung (D), and kidney (E)

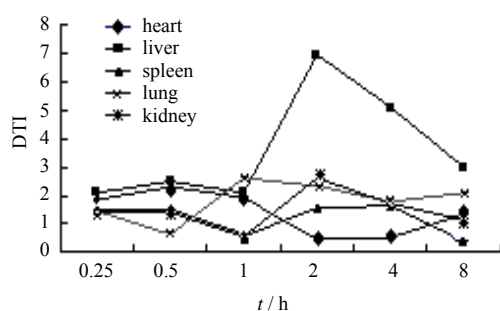


Fig. 4 DTI of BSLNs in various tissues

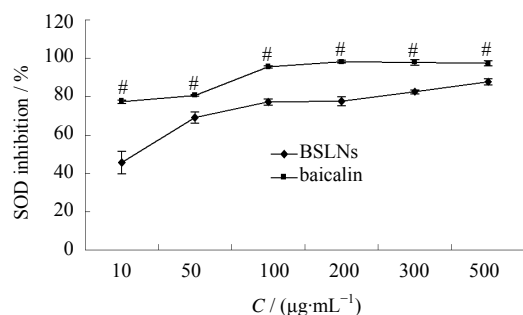


Fig. 5 SOD inhibitory rates of BSLNs and baicalin ($n = 5$)
$P < 0.01$ vs BSLNs group

rate of BSLNs (50 $\mu\text{g}/\text{mL}$) was up to 80.6%, which showed that baicalin had a strong anti-oxidative activity.

Reducing ability Results of reducing ability of three groups were shown in Fig. 6, and the reducing ability of BSLNs was stronger than that of baicalin. With the concentration increasing, the reducing ability was strengthened gradually. But the reducing ability of

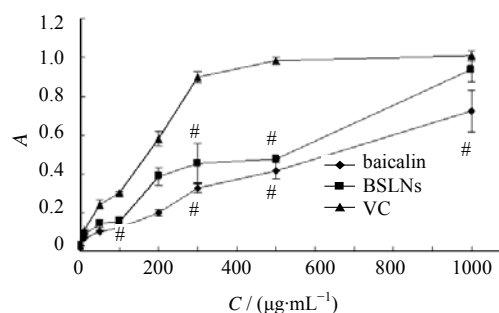


Fig. 6 Reducing abilities of BSLNs, baicalin, and VC
$P < 0.01$ vs VC group

BSLNs and baicalin are both lower than that of VC.

Hydroxyl radical scavenging activity

The results of hydroxyl radical scavenging of three groups were shown in Fig. 7, the scavenging of BSLNs is stronger than that in the control group in every time point with the significant difference.

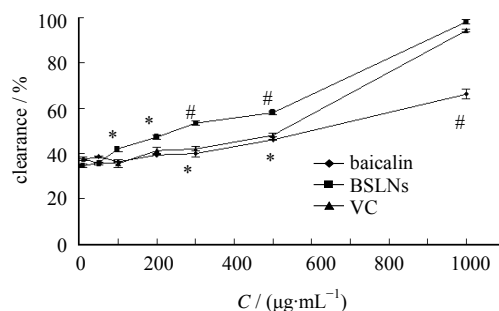


Fig. 7 Hydroxyl radical scavenging activity of BSLNs and baicalin
* $P < 0.05$ # $P < 0.01$ vs VC group

Discussion

In this study, the BSLNs were prepared by ultrasonic emulsification dispersion method, particle size could be achieved within the diameter of 100 nm compared with other methods. It is in order to comply with the requirements of the liver targeting.

In this experiment, SOD inhibitory rate of BSLNs is higher than that of baicalin, the reason may be related to soy lecithin in the excipient, because soy lecithin also has anti-oxidation.

Conclusion

BSLNs are prepared by emulsification ultrasonic dispersion method and the mean particle size is (68.6 ± 8) nm. The EE and DLE are 84.7% and 5.65%, respectively. Targeting and *in vitro* anti-oxidant research indicate BSLNs have significant liver targeting, strong anti-oxidative, and hydroxyl radical scavenging

abilities. These results could provide the basis for the treatment of hepatitis B and liver fibrosis. The pharmacodynamic characteristic needs to be further studied.

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