Anti-hepatocarcinoma effects of chrysin loaded solid lipid nanoparticle against H22 tumor bearing mice

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Abstract Hepatocarcinoma, a malignant cancer, threaten human life badly. It is a current issue to seek the effective natural remedy from plant to treat cancer due to the resistance of the advanced hepatocarcinoma to chemotherapy. Chrysin (Chr), a major symbol ingredient in Chinese Propolis, has a wide range of pharmacological properties and is considered to have anti-hepatocarcinoma effects. However its low oral bioavailability restricts its wide application. In this report, Chr-loaded solid lipid nanoparticles (Chr-SLN) composed of Chr, cremophor EL and glyceryl behenate were prepared by high pressure homogenization technique. The *in vivo* anti-hepatocarcinoma effects of Chr-SLN relative to efficacy of bulk Chr were evaluated. The particle size and zeta potential of Chr-SLN were 479.7 nm and −26.3 mV, respectively. The results showed higher antitumor efficacy against H22 solid tumor bearing mice. These results suggest that the delivery of Chr-SLN is a promising approach for treating tumors.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the third most common cause of cancer death [1]. In last decades, most patients diagnosed with hepatoma have low recovery rates, and conventional and modified therapies currently available are rarely beneficial [2]. Moreover, the limited responses of hepatoma, mainly hepatocellular carcinoma, to these agents are often due to its multidrug resistance to them. Thus, developing new therapeutic agents for hepatocellular cancer becomes an urgent need to reduce the mortality caused by this disease [3]. At present, the demands for more effective and safer therapeutic agents for cancer have greatly increased. Natural products from medical plants are valued as an important source to find innovative agents for treatment of cancer [4].

Chrysin (Chr, Fig. 1), a major symbol ingredient in Chinese propolis, which comes from the dried secreta of *Apid mellifera* L. in accordance with Chinese Pharmacopeia [5]. Chr has been shown to exert a variety of pharmacological effects such as antioxidant, antidiarrheal and anti-inflammatory activities. Chr is a natural flavonoid currently under investigation due to its important biological anti-cancer properties including effects on leukemia [6, 7], cervical [8], oesophageal [9, 10], prostate [11], breast [12, 13], lung epithelial [14, 15], thyroid [16, 17], and hepatocarcinoma [18] cancers.

However, Chr is hardly water-soluble and its absorption *in vivo* is very poor after oral administration [19]. A compound as a drug should have favorable absorption, distribution, metabolism, excretion and toxicity (ADMET) characteristics. To circumvent these pitfalls, several

strategies like cyclodextrin nanoparticles [20] and chemically modified prodrugs [21], have been proposed to deliver Chr in the last few decades.

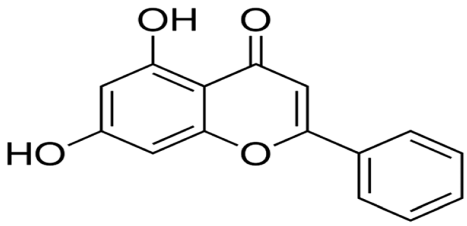

Fig.1 Chemical structure of Chr

Solid lipid nanoparticles (SLN) combine the advantages and avoid the disadvantages of other colloidal carriers, such as liposomes. Proposed advantages include: possibility of controlled drug release and drug targeting, increased drug stability, high drug payload, incorporation of lipophilic and hydrophilic drugs feasible, no biotoxicity of the carrier, avoidance of organic solvents, no problems with respect to large scale production and sterilization [22]. General ingredients of SLN include solid lipid(s), emulsifier(s) and water. The term lipid is used here in a broader sense and includes triglycerides (e.g. tristearin), partial glycerides (e.g. Imwitor), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate). All classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently [22]. In the following years, it has been demonstrated that HPH is a more effective method for the production of submicron sized dispersions of solid lipids compared to high shear mixing or ultrasound. Dispersions obtained in this way are called SLN™. Most SLN dispersions produced by HPH are characterized by an average particle size below 500 nm and a low microparticle content. Other production procedures are based on the use of organic solvents (HPH/solvent evaporation) or on dilution of microemulsions [22, 23]. Among these techniques, the HPH method with a high productivity and a lower level contamination which is favorable for implementation of industrial products has shown great superiority over other methods. In this study, Chr-loaded solid lipid nanoparticles (Chr-SLN) composed of Chr, Cremophor EL and glyceryl behenate were prepared by HPH. The *in vivo* anti-hepatocarcinoma effects of Chr-SLN relative to efficacy of bulk Chr were evaluated.

Materials and Methods

Materials

Chr form was purchased from Aladdin industrial corporation (Shanghai, China). Chr standard was purchased from the National Institutes for food and drug Control (≥98.0%). Glyceryl behenate (Compritol 888 ATO, GATTEFOSSE SAS, France), and Cremophor EL (Kolliphor EL, BASF, Germany).

Male and female kunming mice, body weights 18-22 g were purchased from the medical experimental animal center of Guangdong Province, China. The Natl. Inst. of Health guidelines for the care and use of laboratory animals were followed in all animal experimental procedures. The animals were allowed free access to food and water at all times and were maintained on a 12 h light/dark cycle in a controlled temperature (20 to 25 $^{\circ}C$) and humidity (50±5 %) environment for 1 wk before use. The H22 mouse liver cancer cells were obtained from Shanghai Inst. for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The H22 cells were isolated from the ascites of Kunming mouse following abdominal injection of the H22 cells (0.5 ml 1×106 cells/ml) for 7 days. The cells were passaged in RPMI 1640 containing 10 % heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 2 mmol/l glutamine in a 5 % CO2/95 % air incubator at 37 ˚C. The cells were subcultured until reaching logarithmic growth phase. The viability of H22 cells, stained with trypan blue, was above 97 %.

Preparation of the test solution

HPH technique was applied to prepare Chr-SLN. Compritol 888 of 1.5 % was heated to about 75 ˚C (above melting point), and cremophor EL (Kolliphor EL, BASF, Germany) of 0.75 % was dissolved in distilled water and heated to the same temperature. The Chr powder of 0.15 % was dispersed in melted Compritol solution using high speed homogenization 5000 rpm for 15 min (IKA T18 basic ULTRA-TURRAX®, Germany). Then the pre-mix was passed through a Lab HPH (APV-2000, Germany), 10 cycles were performed at 500 bar, and 20 cycles at 1500 bar.

Characterization of the Chr-SLN

The particle size, polydispersity index (PDI), and Zeta potential measurements were performed on a Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) thermostated at 25 ˚C. The sample was diluted 50 times with bidistilled water before the measurements. All values were measured at an analysis angle of 90 ˚C in a 10-mm diameter cell. Each value reported is the average of three measurements.

Determination of *in vivo* antitumor effect

Antitumor activity against a solid tumor mass was evaluated in Kunming mice. Ten days after receiving tergal s.c. inoculation of 1×10^6 H22 cells prepared as described above. The H22 cell suspension was inoculated to the right armpit of the mice subcutaneously for 0.2 ml per mouse on day 0. The tumor-bearing mice were divided into five groups (10 mice each group), including negative control group (normal saline), positive control group [cyclophosphamide (CTX) 60 mg/kg], and two groups for Chr-SLN and bulk Chr administration with dosages of 60 mg/kg. After administered orally by gastric intubation once a day for continuous 21 days, the mice were sacrificed, and solid tumors were excised and weighed. The antitumor activity was expressed as following formula:

Inhibition rate (%) = [1−(tumor weight of experimental group / tumor weight of control group)] ×100

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Student's t-test was used to compare the mean differences between samples using the statistical software SPSS version 16.0 (SPSS, Chicago). In all cases $P < 0.05$ was considered statistically significant.

Results and Discussion

Particle size analysis and Zeta potential of Chr-SLN

The mean particle size and PDI were measured immediately after the preparation of the NS. The mean particle size with PDI 0.524 was 479.7 nm (Fig. 2 left). The PDI is a measure of particles size distribution. The values less than 0.3 indicate a high degree of homogeneity in particle size and vice versa. The zeta potential of Chr-NS was −26.3 mV (Fig. 2 right).

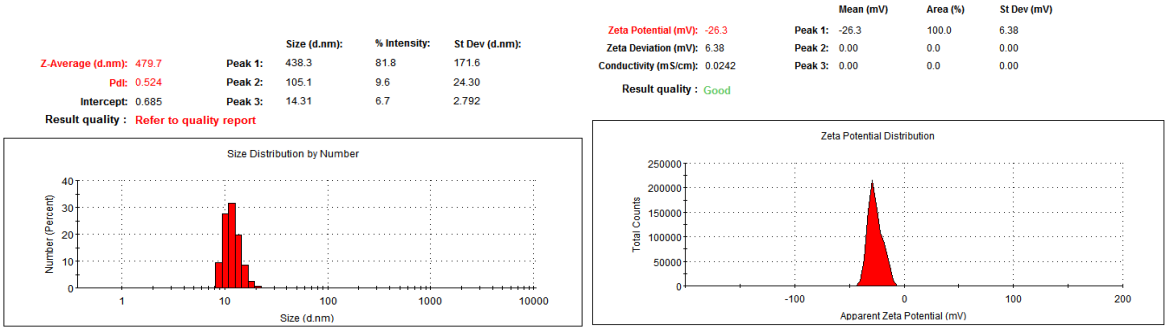

Fig. 2 The particles size and zeta potential of Chr-SLN

Antitumor activity of Cur-SLN on solid tumor *in vivo*

H22 tumor-bearing mice were used to evaluate the antitumor activity of Chr-SLN *in vivo*. Due to the fast growth of tumor, the transplanted tumor model mice in the control group gradually exhibited a series of weak appearance, such as the lost of appetite, the reduced activity and the body

weight with dim hairs. After treated orally with Chr-SLN and bulk Chr, the growth of H22 tumors in the model mice was significantly suppressed compared with control group ($p<0.05$). Inhibiting ratios were 51.1 % and 29.8 % at concentrations of 60 mg/kg body weight, respectively (Tab. 1), which indicated Chr-SLN possessed excellent antitumor activity. Furthermore, the body weights of Chr-NS-treated group were increased significantly when compared with the negative control group during the 21 day experimental period. The frequently used chemotherapy drug CTX, exhibited a high antitumor activity (63.0 %). However, CTX considerably reduced the body weight of tumor-bearing mice, indicating the strong side effect to the body.

Tab. 1 *In vivo* antitumor activities of Chr-SLN on H22-bearing mice

Sample	Dose (mg/kg)	Mice		Body weight (g)		Inhibition rate (%)	*P* value
		Before	After	Before	After		
Control		10	8	31.7±5.2	35.5±8.9		
CTX	60	10	6	32.6±5.9	21.9±4.8	63	0.004
Chr-SLN	60	10	10	30.7±3.1	32.7±3.5	51.1	0.076
Chr	60	10	8	29.9±3.3	30.8±3.1	29.8	0.047

Conclusion

In present study, we demonstrated that Chr-SLN effectively inhibited the growth of H22 tumor bearing mice. Moreover, Chr-SLN produced less adverse effects compared with CTX, greatly prolonged the life span of tumor-bearing mice. Therefore, Chr-SLN may be explored as a novel potential antitumor agent for the functional food and pharmaceutical purpose. This study also provides evidences to support the therapeutic effects of compound for treatment of cancer in China. Despite of the promising results from our current investigation, there are still a plethora of practical issues which may be difficult to reconcile for the ultimate use of Chr-SLN for the novel target-therapy in cancer management.

Acknowledgments

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