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Research Article

Anti-hepatocarcinoma Effects of a Food Additive Resveratrol Nanosuspension Against Human HepG2 Cells

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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. Resveratrol (Res), a major symbol ingredient in red grapes and peanuts, 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, Res-nanosuspension (Res-NS) composed of Res and poloxamer 188 was prepared by high pressure homogenization technique. The in vitro anti-hepatocarcinoma effects of Res-NS relative to efficacy of bulk Res were evaluated. The particle size and zeta potential of Res-NS were 159.4 nm and -2.1 mV, respectively. MTT assay showed that Res-NS effectively inhibited the proliferation of HepG2 cells and the corresponding IC_{50} values of Res-NS and bulk Res were 2.91 and 7.13 μg/mL. These results suggest that the delivery of Res-NS is a promising approach for treating tumors.

Keywords: Antitumor activity, cytotoxicity, HepG2 cells, nanosuspension, resveratrol

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the sixth most common cancer in the world and the third most common cause of cancer death (Parkin, 2001). In last decades, most patients diagnosed with hepatoma have low recovery rates and conventional and modified therapies currently available are rarely beneficial (Thomas and Zhu 2005). 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 (Deng *et al*., 2006). 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 (Liang *et al*., 2012).

Resveratrol (3, 5, 4'-trihydroxystilbene, Res, Fig. 1), a major symbol ingredient in red grapes and peanuts (Chong *et al*., 2009; Sales and Resurreccion 2014). Res was first isolated from the roots of white hellebore in 1940 in Japan and later found in traditional Chinese medicine (Sinclair and Baur, 2006). It was initially characterized as a phytoalexin (substance produced by

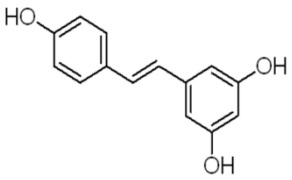

Fig. 1: Chemical structure of Res

higher plants in response to attack by pathogens such as bacteria and fungi, or stress) and achieved notoriety in the scientific literature in 1992, when it was postulated as being responsible for the cardiac protective effects of wine (effect called "French paradox") (Renaud and de Lorgeril, 1992). Since then, Res has been shown to exert a variety of pharmacological effects such as antioxidant, antidiabetes, anti-inflammatory and anti-cancer activities. Res is a natural compound currently under investigation due to its important biological anti-cancer properties, including effects on leukemia, skin, breast, lung gastric, colorectal, neuroblastoma, pancreatic and hepatoma cancers (Athar *et al*., 2007; Bishayee *et al*., 2010; Carter *et al*., 2014; Hiroto *et al*., 2011; Rajasekaran *et al*., 2011; Schuster *et al*., 2014; Weng *et al*., 2010; Zhang and Yang, 2014).

However, Res is hardly water-soluble and its absorption *in vivo* is very poor after oral administration

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(Franciosoa *et al*., 2014). A compound as a drug should have favorable Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) characteristics. To circumvent these pitfalls, nanomedicine have been proposed to deliver Res in the last few decades (Amiot *et al*., 2013; Aras *et al*., 2014; Sessa *et al*., 2014; Pandita *et al*., 2014).

Nanocrystal suspension, nanosuspension (NS) for short, is a carrier-free nanoparticle system containing only pure drug crystal and minimum surfactant and/or polymer for stabilization (Gao *et al*., 2013). Reduction of particle size by nanocrystal technology to the nano-scale usually leads to a significant increase in drug solubility and dissolution rate with an obvious improvement in drug bioavailability (Liversidge and Cundy, 1995). Liversidge and Cundy (1995) reported that, in the same dosage, danazol NS with the average particle size of 169 nm could obtain the C_{max} as high as 3.01 mg/mL and the bioavailability of 82% in beagle dogs, while the commercially available danazol suspension with the average particle size of 10 mm could only obtain the C_{max} of 0.20 mg/mL and the bioavailability of 5 %. It could be found that NS significantly enhanced the oral absorption of danazol, a poorly water-soluble drug. A few techniques have been used to prepare drug loaded NS, including nanoprecipitation, pearl-milling, high speed homogenization, sonication and High-Pressure Homogenization (HPH) (Zhang *et al*., 2007; Karadag *et al*., 2014). 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, we evaluate the human HepG2 cells anti-hepatocarcinoma activity of Res-NS relative to efficacy of bulk Res delivery.

MATERIALS AND METHODS

Materials: Res form was purchased from Aladdin industrial corporation (Shanghai, China). Res standard was purchased from the National Institutes for food and drug Control (≥98.0%). Poloxamer 188 (P188, Lutrol® F68) was kindly donated from BASF (Ludwigshafen, Germany). MTT was purchased from Sigma Chemical (St. Louis, MO).

Preparation of the test solution: HPH technique was applied to prepare Res-NS. Briefly, P188 of 1.0% was dissolved in distilled water. The Res powder of 0.05% was dispersed in the aqueous surfactant solution using high speed homogenization 5000 rpm for 10 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 Res-NS: 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.

Cell viability assay: Cells were treated with different concentrations (0, 0.1, 1, 10 and 100 μg/mL) of Res solution and Res-NS, respectively. And then, the effect of Res-NS on the viability of cells was determined by the colorimetric MTT assay. The inhibition rate was expressed as following formula:

Inhibition rate (%) = [1-(absorbance of experimental group/absorbance 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 Res-NS: The mean particle size and PDI were measured

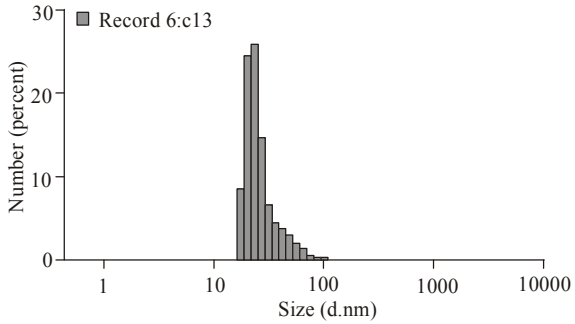

Fig. 2: The particles size of Res-NS

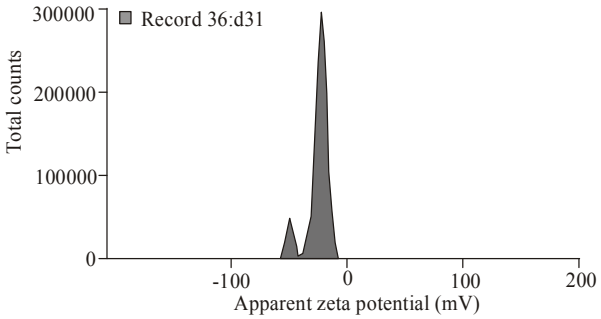

Fig. 3: The zeta potential of Res-NS

immediately after the preparation of the NS. The mean particle size with PDI 0.421 was 159.4 nm (Fig. 2). 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 Res-NS was -22.1 mV (Fig. 3).

Cytotoxicity of Res-NS: To determine whether Res-NS has growth-inhibitory effects, HepG2 cells were exposed to different concentrations of Res-NS for 72 h. The data showed that the growth of human HepG2 cells were significantly inhibited by Res-NS, the IC_{50} of Res-NS and Res solution were 2.91 and 7.13 μg/mL, respectively.

CONCLUSION

In present study, we demonstrated that Res-NS effectively inhibited the growth of HepG2 cells *in vitro*. Therefore, Res-NS 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 Res-NS for the novel target-therapy in cancer management.

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