Anti-hepatocarcinoma Effects of Resveratrol Loaded Solid Nanodispersion by a New Material Nano Silica

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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-solid nanodispersion (Res-SND) composed of Res, poloxamer 188 and nano silica was prepared by high pressure homogenization and sieving methods. The *in vitro* anti-hepatocarcinoma effects of Res-SND relative to efficacy of bulk Res were evaluated. The particle size and zeta potential of Res- SND were 198.6 nm and −14.2mV, respectively. MTT assay showed that Res-SND effectively inhibited the proliferation of HepG2 cells, and the corresponding IC_{50} values of Res-NS and bulk Res were 2.23 and 7.13 μg/ml. These results suggest that the delivery of Res-SND 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].

Resveratrol (Res, Fig. 1), a major symbol ingredient in red grapes and peanuts[5, 6]. Res was first isolated from the roots of white hellebore in 1940 in Japan, and later found in traditional Chinese medicine[7]. It was initially characterized as a phytoalexin (substance produced by 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")[8]. 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[9-13].

However, Res is hardly water-soluble and its absorption *in vivo* is very poor after oral administration[14]. A compound as a drug should have favorable absorption, distribution, metabolism, excretion and toxicity characteristics. To circumvent these pitfalls, nanomedicine have

been proposed to deliver Res in the last few decades[15-18]. Nanonization, the production of drug nanocrystals, is a common approach to overcome poor drug solubility in water. Nanonization produces drug particles in the sub-micron range via either bottom-up methods such as precipitation and self-assembly or top-down technologies such as milling and high pressure homogenization[19,20]. To stabilize nanocrystal formulations, hydrophilic polymers with or without surfactants are added to the nanocrystal suspensions. Nanonization dramatically increases the drug particle surface area, thereby enhancing the rate of dissolution. In addition, increase in saturation solubility can also occur as described by Ostwald-Freundlich's equation[21]. Nanocrystal formulations have been reported to enhance the oral exposure by up to 60-fold, when compared to micronized formulation of the same drug substance[22].
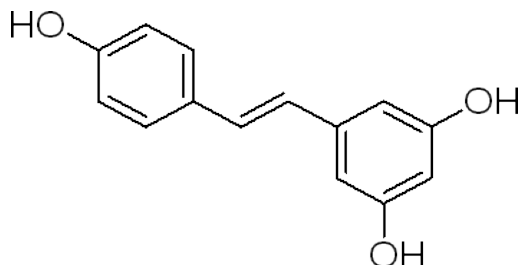

Fig.1 Chemical structure of Res

Solid dispersions of drugs molecularly dispersed in hydrophilic carriers have been extensively studied to improve the bioavailability of poorly soluble compounds. Current solid dispersion techniques including spray-dried dispersion and hot-melt extrusion, convert crystalline drug into the amorphous form. The resulting high surface energy form leads to increase in solubility and the rapid dissolution of the hydrophilic carrier matrix exposes the dispersed drug. Generally for drugs that are dissolution rate-limited, even a small decrease in particle size results in substantial gains in oral exposure[23]. Among the various techniques to produce solid dispersions, melting and solvent evaporation are the principal methods[24]. Besides increasing dissolution and bioavailability, other biopharmaceutics benefits of solid dispersions include greater reproducibility of oral absorption and improved dose–bioavailability proportionality.

While nanocrystal and solid dispersion platforms have existed for some time and provide advantages, several limitations have prevented their routine use in current marketed products. So far, only six commercial products, namely Rapamune (sirolismus, former Wyeth), Emend (aprepitant, Merck), TriCor (fenofibrate, Abbott), Megace (megestrol acetate, Par Pharmaceutical), Invega Sustenna (paliperidone palmitate, Janssen) and Triglide (fenofibrate, Skye Pharma) have resulted from nanocrystal technology and approximately ten solid dispersion products are commercially available[19]. The sparse use of nanocrystal and solid dispersion technologies are due at least in part to their stringent process requirements and often harsh treatment conditions involving heating and cooling cycles. Furthermore, the chemical and physical stability of products produced this way is a major concern. Challenges in scale up, and manufacturing costs are also of consideration.

For production of nanocrystals, the most commonly used techniques are precipitation, pearl milling and high pressure homogenization[24]. The precipitation method involves dissolving the drug in a solvent and then adding to a non-solvent, which leads to the production of finely dispersed, precipitated drug. The application of this method is limited by the use of solvents, which is problematic for newly developed drugs that are poorly soluble in both aqueous and organic solvents. The use of solvents also necessitates a secondary drying step to remove the residual solvent, which increases complexity as well as cost. Furthermore, the size of nanocrystals is often hard to control and scale up is difficult due to the lack of defined parameters that control the process. The stability of nanocrystals is a major concern; particle aggregation, particle growth and form change can easily occur without the proper process controls in place[25]. Similarly, current top-down methods such as pearl milling and high pressure homogenization have limitations such as the need for repeated milling cycles, as well as the potential for contamination from erosion of milling materials. High pressure homogenization in particular requires a relatively high number of cycles to achieve

sufficient particle size reduction, which increases cost and risk of contamination and product degradation. As in the case of the bottom-up approach, the resulting suspension is not readily amenable to solid dosage form manufacturing without additional drying operations.

Solid dispersions are most commonly prepared by the melting (fusion) or the solvent method. In the melting method, components are heated above their melting or glass transition temperatures followed by mixing and cooling. Hot melt extrusion is a scalable variation of this method. Another application in this category is spray congealing. Draw-backs of the melting method are potential thermal degradation, sublimation and polymorphic modifications. Furthermore, miscibility gaps in the liquid state influence the degree of dispersion in the solid state, which may lead to phase separation and crystallization. With the solvent method, solid dispersions are obtained by evaporating the common solvent from drug-carrier solution. Commonly used techniques include spray drying and freeze drying. The use of organic solvent imposes restrictions on the physicochemical properties of the drug and narrows the applicability of this method, since many new compounds in the development pipeline also have limited solubility in organic solvents. What's more, finding a common solvent for both carrier and drug is not always simple and straightforward. In addition, a secondary drying step is imperative to reduce residual solvent below toxicity level. Also the residual solvent can cause chemical stability problems as well as affect the physical stability of the solid dispersion matrix by acting as a plasticizer[19].

In order to overcome the limitations associated with producing nanocrystals and traditional solid dispersions, as well as address the need to enhance the aqueous solubility of poorly water-soluble drugs. The solid nanodispersion (SND) technology combines advantages of drug nanocrystals and solid dispersion technologies, while addressing inherent limitations of the nanocrystal and solid dispersion methods. Silica nanoparticles (Nano silica), a new materials are amorphous in nature. The nanoparticles are small in size and have a large specific surface area, which improves drug dispersion in carriers, and significantly decreases the particle size of the drug. Silica-based nanoparticles for solid dispersion applications have been previously studied [26, 27]. In the present study, we chose to use nano silica, since it present some of the best results. Res-SND composed of Res, poloxamer 188 and nano silica was prepared by HPH and sieving methods and evaluate the human HepG2 cells anti-hepatocarcinoma activity of Res-SND 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). Nano silica (TH 99) with a primary particle size of about 120-180 nm was kindly supplied by Xingtai TianHe Non-metal Material Co., Ltd. (Xingtai, China).

Preparation of solid nanodispersion and physical mixture

Physical mixture To ensure a uniform product, the geometric dilution method was used to prepare Res-SND. Briefly, Res of 15.0 %, P188 of 1.2 % and nano silica of 83.8 % was carried out using geometric dilution, then sieving through a 0.25 mm mesh and storage in airtight glass desiccators under a vacuum.

Res-SND High pressure homogenization technique was applied to prepare Res-SND. Briefly, P188 of 1.2 % was dissolved in distilled water and heated to about 70 ^{0}C and added Res powder of 15.0 %. The solution using high speed homogenization 5000 rpm for 15 min (IKA T18 basic ULTRA-TURRAX®, Germany), and passed through a Lab high pressure homogenization (APV-2000, Germany), 10 cycles were performed at 500 bar, and 20 cycles at 1500 bar, then added nano silica of 83.8 %, kneading and sieving through a 0.25 mm mesh, vacuum drying (70 ^{0}C) 2 h and storage in airtight glass desiccators under a vacuum.

Characterization of the Res-SND

The particle size, polydispersity index (PDI), and Zeta potential measurements were performed on a Nano-ZS90 (Malvern Instruments Ltd., Malvern, UK) thermostated at 25 ^{0}C. The sample was diluted 50 times with bidistilled water before the measurements. All values were measured at an analysis angle of 90 ^{0}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-SND respectively. And then, the effect of Res-SND 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

Physicochemical characterization of Res-SND

The mean particle size and PDI were measured immediately after the preparation of the SND. The mean particle size with PDI 0.636 was 198.6 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 Chr-NS was −14.2 mV (Fig. 3).
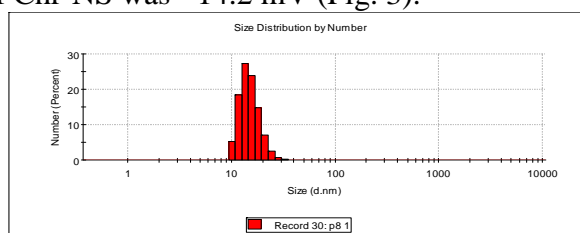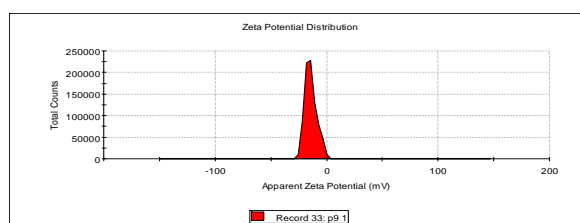

Fig. 2 The particles size of Res-SND

Fig. 3 The zeta potential of Res-SND

Cytotoxicity of Res-SND

To determine whether Res-SND has growth-inhibitory effects, HepG2 cells were exposed to different concentrations of Res-SND for 72 h. The data showed that the growth of human HepG2 cells were significantly inhibited by Res-SND, the IC$_{50}$ of Res-SND and Res solution were 2.23 and 7.13 µg/ml respectively.

Conclusions

In present study, Res-SND composed of Res, poloxamer 188 and nano silica was successfully prepared by HPH and sieving methods, and demonstrated that Res-SND effectively inhibited the growth of HepG2 cells *in vitro*. Therefore, Res-SND 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-SND for the novel target-therapy in cancer management.

Acknowledgment

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