RESEARCH ARTICLE

Effect of Paclitaxel-loaded Nanoparticles on the Viability of Human Hepatocellular Carcinoma HepG2 Cells

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Abstract

Objective: To explore effects of paclitaxel-loaded poly lactic-co-glycolic acid (PLGA) particles on the viability of human hepatocellular carcinoma (HCC) HepG2 cells. Materials and Methods: The viability of HepG2 cells was assessed using MTT under different concentrations of prepared paclitaxel-loaded particles and paclitaxel (6.25, 12.5, 25, 50, and 100 mg/L), and apoptosis was analyzed using Hochest33342/Annexin V-FITC/PI combined with an IN Cell Analyzer 2000. Results: Paxlitaxel-loaded nanoparticles were characterized by narrow particle size distribution (158.6 nm average particle size). The survival rate of HepG2 cells exposed to paclitaxel-loaded PLGA particles decreased with the increase of concentration and time period ($P<0.01$ or $P<0.05$), the dose- and time-dependence indicating sustained release ($P<0.05$). Moreover, apoptosis of HepG2 cells was induced, again with an obvious dose- and time-effect relationship ($P<0.05$). Conclusions: Paclitaxel-loaded PLGA particles can inhibit the proliferation and induce the apoptosis of HCC HepG2 cells. This new-type of paclitaxel carrier body is easily made and has low cost, good nanoparticle characterization and sustained release. Hence, paclitaxel-loaded PLGA particles deserve to be widely popularized in the clinic.

Keywords: Paclitaxel-loaded nanoparticles - HCC - HepG2 cells - viability - apoptosis

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Introduction

Paclitaxel, a kind of diterpenoid compound and complex secondary metabolite derived from the genus of taxus chinensis, is a broad-spectrum chemotherapeutic drug highly effective against cancer found in the 20th century (Dong et al., 2012; Xu et al., 2012). It is a highly effective tubulin inhibitor, different from the targets and mechanisms of microtubule inhibitors. That is, it can influence the dynamic equilibrium between microtubule polymerization and depolymerization, hinder the formation of spindle fibers and block the cell cycle in phase G2/M by coupling specific loci of cell microtubules, promoting microtubule polymerization, making the microtubule structure stable and blocking the depolymerization into subunits, consequently resulting in cell apoptosis and death (Symmans et al., 2000; Ma et al., 2010).

At present, paclitaxel is clinically used as the first-line treatment for breast cancer, lung cancer, esophageal cancer, colon cancer, lymphoma, acute leukemia, melanin, ovarian cancer and gastric cancer (Pitakkarnkul et al., 2013; Jianmin et al., 2014; Tanaka et al., 2014; Feng et al., 2015; Langer et al., 2015). However, it is hard to be absorbed by taking orally due to its physicochemical property, such as poor water solubility, so it is mostly made into injection. Nevertheless, polyoxyethylene castor oil injection presently used in clinic causes many severe adverse reactions, such as hypersensitivity, toxic renal damage, neurovirulence and cardiotoxicity. To solve this problem, nanocarrier is attracted widespread attention (Chao et al., 2007; Gong et al., 2009). In this study, paclitaxel-loaded poly lactid-co-glycolic acid (PLGA) nanoparticles were prepared, and their inhibitory and apoptotic effects on human hepatocellular carcinoma (HCC) HepG2 cells were observed.

Materials and Methods

Key reagents and equipments

Laser particle analyzer was purchased from British Malvern Company. Transmission electron microscopy (TEM) and scanning electron microscope (SEM) were provided by Japanese Hitach Company. Probe-type ultrasonic instrument was purchased from American Sonic and Materials Company, inverted fluorescence microscope from Leica Company, IN Cell Analyzer 2000 from American GE Company, CO_2 cell culture box from Heal Force Bio-meditech Holdings Group, paclitaxel API from Wuhan Hezhong Bio-Chemical Manufacture Co., Ltd, DAPI and MTT from Sigma Company and Annexin V-FITC Apoptosis Assays Kit from Beyptime. Both

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dichloromethane and polyvinyl Acetate were provided by Tianjin Damao Chemical Reagent Factory.

Preparation of paclitaxel-loaded PLGA nanoparticles

Paclitaxel powder (30 mg) and PLGA (100 mg) were weighed and dissolved into dichloromethane solution. After completely dissolved, the solution was injected in 1% polyethylene aqueous solution, performed with ultrasonic dispersion for 5 min under the condition of ice bath, and then formed oil-in-water emulsion. The solution was stirred for 4 h in fuming cupboard under the normal pressure until the organic solvent was removed completely, and centrifuged at 23 000 r/min for 20 min. The solidified nanoparticles were collected, washed by distilled water and centrifuged for 3 times. After freeze drying, paclitaxel-loaded PLGA nanoparticles were prepared successfully and preserved at 4°C.

Surface characterization of paclitaxel-loaded PLGA nanoparticles

The surface characterization was described using laser particle analyzer, TEM and SEM. Particle size determination: A small number of prepared paclitaxel-loaded PLGA nanoparticles were dissolved in double distilled water (as solvent) and mixed evenly to make the solution clear, and without coalescence particles. The particle size distribution was detected using particle size instrument. Paclitaxel-loaded nanoparticles observed under SEM: A small number of paclitaxel-loaded nanoparticles were blown by rubber pipette bulb onto double stick tape which was adherent to sample stage and given metal spraying and finally observed under SEM. Paclitaxel-loaded nanoparticles observed under TEM: A small number of paclitaxel-loaded nanoparticles were dispersed evenly into double distilled water, then dripped into 300-mesh copper net and determined after natural drying.

Cell culture

Human HCC cell line HepG2 was purchased from Cell Resource Center, Shanghai Institutes for Biological Sciences. HepG2 cells were inoculated into RPMI 1640 culture medium which contained 10% of fetal bovine serum (FBS), 100 U/mL of penicillin and 100 mg/L of streptomycin, and then the culture medium was placed in the incubator with 5% CO_2 at 37°C. Culture solution was exchanged every 1~2 d. After they grew against the wall, HepG2 cells were digested by 0.25% of trypsase for sub-culturation. Cells in logarithmic phase were collected for ensuing experiment.

Cell viability detected by MTT

HepG2 cells in logarithmic phase were inoculated into 96-well plate by 5×104/well, 100 uL/well. Experimental group and control group were designed, respectively added with paclitaxel-loaded nanoparticles and paclitaxel at different concentrations (6.25, 12.5, 25, 50, and 100 mg/L) and cultured for 24, 48 and 72 h. Each well was added with 50 μL of MTT solution (5 g/L) and continued to incubate for 4 h at 37 °C. After centrifugation for 5 min, the culture medium was removed and each well was added with 150 μL of DMSO particles for shaking until the particles were completely resolved. A microplate reader was used to detect the optical density (OD) at the wavelength of 492 nm to observe the viability of HepG2 cells. Cell viability (%)= (OD of experimental group/OD of control group)×100%. The experiment was repeated 3 times for average one.

Detection of cell apoptosis

HepG2 cells in logarithmic phase were incubated in 96-well plate in a 5% CO_2 incubator at 37 °C. Each well was 100 μL, containing 5 000~7 000 cells. Drug toxicity and dose dependence were observed. Paxlitaxel nanoparticles at different concentrations (12.5, 25, 50 and 100 mg/L) were added in experimental group, while 0.1% DMSO solution in control group. After incubation for 24, 48 and 72 h, 30 μL of 2.5% Annexin V-FITC binding buffer was added, shaked slightly and incubated for 10 min at room temperature away from light. Then the supernatant was removed and 30 μL of Annexin V-FITC binding buffer with 5% propidium iodide was added for ice-bath incubation for 10 min away from light. Afterwards, the supernatant was removed again and cells were washed by PBS twice, added with 50 μL of 10 mg/L Hochest33342 for incubation for 10 min away from light and washed by PBS twice at 4 °C. Finally, each well was added with 150 μL PBS. Finally, the apoptosis of HepG2 cells was detected using IN Cell Analyzer 2000.

Statistical data analysis

SPSS13.0 software package was used to analyze the data. Measurement data were compared with t test, expressed as the mean±standard deviation (x±s). A value of $P<0.05$ was considered to be statistically significant.

Results

Surface characterization of paclitaxel-loaded nanoparticles

Paxlitaxel-loaded nanoparticles were characterized by narrow particle size distribution, with the average particle size being 158.6 nm, ranged 78.0~178.8 nm (Figure 1). TEM showed paclitaxel-loaded nanoparticles had smooth surface, uniform particle size and sphere-like shape. SEM analysis showed that paxlitaxel-loaded nanoparticles were like good solid balls, with the particle size being 163.69 nm and 92.26 nm respectively (Figure 2~3).

Effect of paclitaxal-loaded nanoparticles and paclitaxal on the viability of HepG2 cells

After intervention for 24, 48 and 72 h, the viability

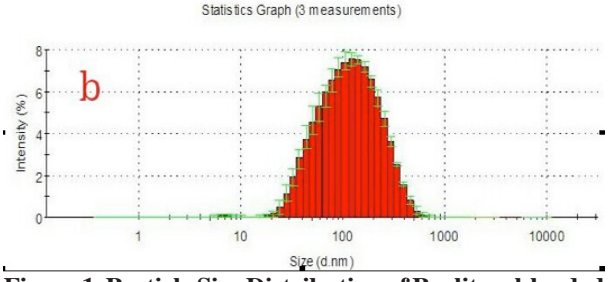

Figure 1. Particle Size Distribution of Paxlitaxel-loaded Nanoparticles

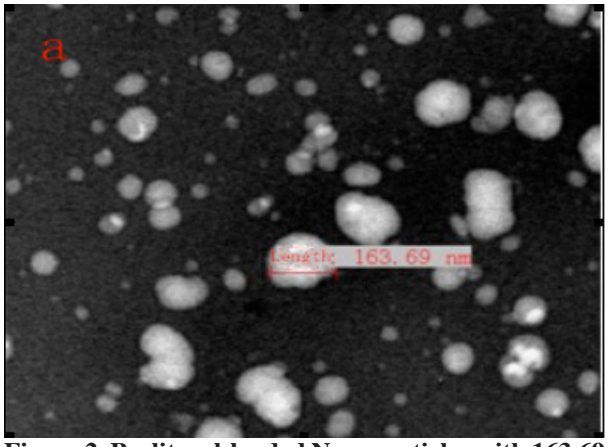

Figure 2. Paclitaxel-loaded Nanoparticles with 163.69 nm of Particle Size

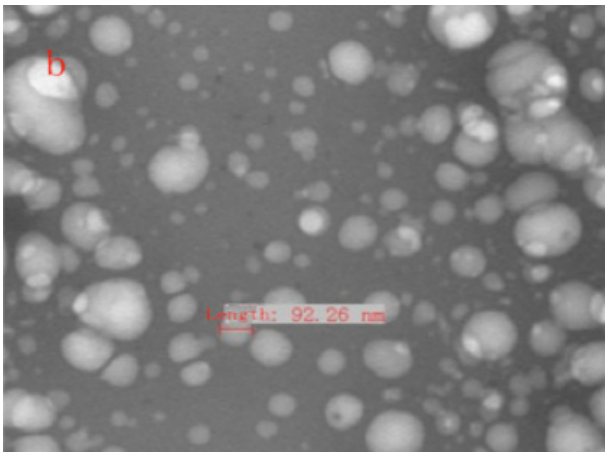

Figure 3. Paclitaxel-loaded Nanoparticles with 92.26 nm of Particle Size

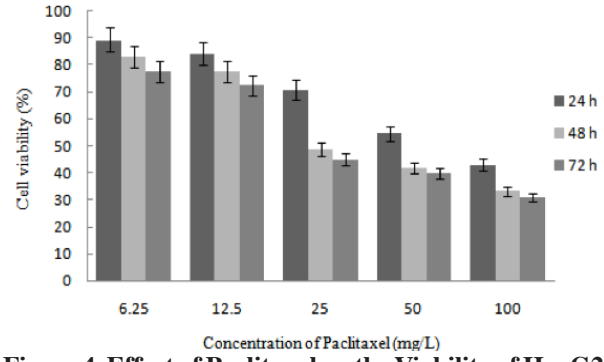

Figure 4. Effect of Paclitaxal on the Viability of HepG2 Cells

and growth condition of HepG2 cells under different concentrations of paclitaxel-loaded PLGA particles and paclitaxel were respectively observed. The survival rate of HepG2 cells intervened by paclitaxel decreased with the increase of concentration ($P<0.05$), but tended to be stable after intervention for 48 h, showing that paclitaxel could inhibit the viability of HepG2 cells and the inhibitory effect increased as the concentration of paclitaxel increased, but nearly reached the peak after intervention for 48 h (Figure 4). However, the survival rate of HepG2 cells intervened by paclitaxel-loaded PLGA particles decreased with the increase of concentration and time extension ($P<0.01$ or $P<0.05$), showing that paclitaxel-loaded PLGA particles suppressed the viability of HepG2, which was in a dose-

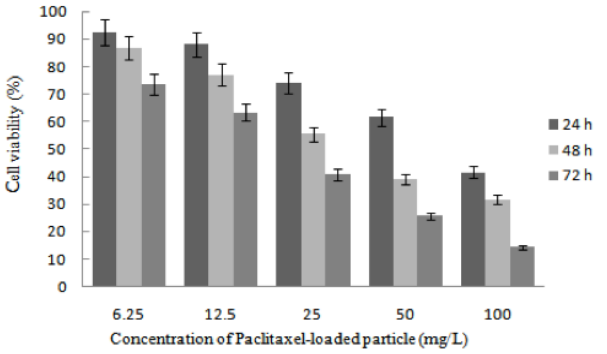

Figure 5. Effect of Paclitaxal-loaded Nanoparticles on the Viability of HepG2 Cells

Table 1. Apoptosis Rates of HepG2 Cells Intervened by Different Concentrations of Paclitaxel-loaded PLGA Particles at Different Time Points (x±s, %)

Groups		Culturetime	Cell apoptosis rate	Necrosis/late apoptosis rate
Control group			2.7±9.8	0.2±0.8
Experimental group	12.5 mg/L	24 h	9.6±3.4*	4.6±0.8**
		48 h	16.3±6.1**	5.0±1.9**
		72 h	28.8±4.6**	8.4±2.1**
	25 mg/L	24 h	14.5±3.1**	5.6±2.7**
		48 h	23.4±5.8**	8.4±3.3**
		72 h	59.1±8.2**	14.6±2.8**
	50 mg/L	24 h	18.6±8.8**	8.4±2.5**
		48 h	44.7±7.3**	13.1±3.2**
		72 h	60.7±7.0**	26.8±6.6**
	100 mg/L	24 h	25.9±9.3**	10.0±4.2**
		48 h	43.8±6.5**	19.4±3.7**
		72 h	61.6±7.8**	31.5±4.4**

Compared with control group, *$P<0.05$, **$P<0.01$

and time-dependence manner. Besides, it also indicated obviously sustained release (Figure 5).

Effect of paclitaxal-loaded nanoparticles on the apoptosis of HepG2 cells

The apoptosis rate of human HCC HepG2 cells intervened by paclitaxel-loaded PLGA particles was analyzed using Hochenst33342/Annexin V-FITC/PI combined with IN Cell Analyzer 2000. Paclitaxel-loaded PLGA particles could induce the apoptosis of HepG2 cells obviously, which was in an obvious dose- and time-effect relationship ($P<0.05$ or $P<0.01$) (Table 1).

Discussion

Many anti-tumor drugs do not work efficiently in treating neoplastic disorders duo to the solubility, stability, metabolism and toxicity to some extent (Qiu et al., 2009; Wang et al., 2013). Paclitaxel is an alkaloid with unique anti-tumor mechanism, effective in multiple advanced tumors and such effects have been proved. At present, many preparations of paclitaxel are mainly based on lipidosomes (Xu et al., 2013). Although lipidosome is as a drug carrier with many advantages, the molecule of natural-phospholipid lipidosome contains unsaturated fatty acid chains which are susceptible to oxidization and hydrolysis, and results in the toxicity caused by

drug leakage, which limits the application of paclitaxel (Danhier et al., 2015). To solve this issue, selection of drug carriers have been attracted widespread attention (Desai et al., 2013). PLGA nonoparticles were applied in this study, and the results showed that PLGA nonoparticles could extend the time of drug effect and release drugs slowly, with high stability in vitro and small toxicity, suggesting that it is an ideal fat soluble drug carrier. Paclitaxel-loaded PLGA particles were prepared easily, with low cost and energy consumption. In this study, paxlitaxel-loaded nanoparticles were characterized by narrow particle size distribution, with the average particle size being 158.6 nm, ranged 78.0~178.8 nm. TEM showed paclitaxel-loaded nanoparticles had smooth surface, uniform particle size and sphere-like shape. SEM analysis showed that paxlitaxel-loaded nanoparticles were like good solid balls.

Multiple studies showed that many chemotherapeutics could inhibit the proliferation and induce cell apoptosis, such as paclitaxel (Yadav et al., 2014; Lim et al., 2015; Lin et al., 2015). By promoting microtubulin to form into canaliculus and suppressing depolymerization, paclitaxel-loaded drugs can lead to aberrant arrangement of microtubule fasolculus, formation of asteriform, abnormal function of spindle apparatus and inhibition of cell mitosis, thus inducing apoptosis and inhibiting cell proliferation (Pan et al., 2013). MTT assay showed that the survival rate of HepG2 cells intervened by paclitaxel decreased with the increase of paclitaxel concentration, but tended to be stable after intervened for 48 h, showing that paclitaxel could inhibit the viability of HepG2 cells and the inhibitory effect increased as the concentration of paclitaxel increased, but nearly reached the peak after intervention for 48 h. However, the survival rate of HepG2 cells intervened by paclitaxel-loaded PLGA particles decreased with the increase of concentration and time extension, showing that the inhibitory effect of paclitaxel-loaded PLGA particles on the viability of HepG2 cells was in a dose- and time-dependence manner and had obviously sustained release.

In conclusion, paclitaxel-loaded PLGA particles can inhibit the proliferation and induce the apoptosis of HCC HepG2 cells. It is a new-type paclitaxel carrier body which is easily to be made and has low cost, good nonoparticle characterization and sustained release. Hence, paclitaxel-loaded PLGA particles deserve to be widely popularized in clinic.

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