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The biological activity of cationic liposomes in drug delivery and toxicity test in animal models

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ABSTRACT

In the study we made use of DOTAP (1,2-dioleoyl-3-trimethylammonium), DOPE (1,2-dioleoyl-snglycero-3-phosphoethanolamine) and PEG-PE (polyethylene glycol- polyethylene) to make cationic PEG-liposomes by ultrasonic dispersion method. The plasmid pGPU6 combined with cationic PEG-liposomes or Liopofectamin 2000 was used to transfect PC3 cells to judge the transfection efficiency. HE staining showed that the pGUP6-shAurora B plasmid/liposomes complex could significantly inhibit tumor growth in mice tumor model. The results indicated that there was no remarkable difference between the homemade liposomes and Lipofectamin 2000 after transfection, with transfection efficiency over 80%. And the homemade liposomes also had high transfection efficiency *in vivo*. No significant side effects were observed on weight, coat condition, behavior or appetite and the life span of mice treated with pGPU6-shAurora B were extended. Beyond that, there were no differences in mortality or in pathological changes to the heart, liver, spleen, lungs and kidneys among all the mice.

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1. Introduction

Cancer is considered as a major cause of mortality, which has been the third most common cause of death in the world only after heart and infectious diseases. Among these, prostatic carcinoma is also a severe health problem threatening people's life. Prostatic carcinoma is greater prevalence in the west (Qiu and Tanaka, 2006). Some positive results on the mechanism and treatment of cancer have been obtained over the past few decades. Although new treatments were continually being found and developed, people were still not completely overcome the problem. Effective cancer therapy remains an important challenge. Despite the development of many effective therapy methods, anticancer drugs have non-ideal properties, such as, low aqueous solubility, irritant properties, lack of stability, rapid metabolism and non-selective drug distribution, which could result in a large amount of side effect (Tila et al., 2015). Hence, the curative effect of cancer therapy is determined by the ability to balance their benefits against their toxicity (Kong et al., 2014; Shao et al., 2013). Likewise, although many gene therapy strategies for prostate cancer have been discovered and trialed, further research is required to improve the drug entrapment. Here, we found a novel liposome, which could achieve satisfactory results.

Liposomes are spherical, self-closed structures formed by lipid bilayer with high biocompatibility (Lee et al., 2013) and low toxicity (Fan et al., 2012). Liposomes, initially called smectic mesophases, were first discovered by Bangham, A.D. and his coworkers, when phospholipids were dispersed in water for electron microscopy in the year of 1965. They observed that phospholipids formed spherical, self-closed vesicles with concentric lipid bilayers and spontaneously formed hydrophilic cores in water (Bangham et al., 1965). Furthermore, people found endothelial cells were bound

Abbreviations: DOTAP, 1,2-dioleoyl-3-trimethylammonium; DOPE, 1,2-dioleoyl-snglycero-3-phosphoethanolamine; PEG-PE, polyethylene glycol-polyethylene; EPR, enhanced permeability and retention; RNAi, RNA interference; Aurora A, Aurora kinase A; Aurora B, Aurora kinase B; Aurora C, Aurora kinase C; HE, Hematoxylin Eosin; MPS, mononuclear phagocyte system.

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together by tight junctions in healthy blood vessels, and formed a single cell layer, which constituted the endothelium. This would be a barrier which can prevent any large particles in the blood from leaking out of the vessels. However, tumor epithelial cells were not closely bound together on account of the defective endothelial cells in tumor, which might result in large loophole and losing their normal barrier function. Nevertheless, liposomes could not enter the endothelium of health tissues, but can only circulate in the blood (Chauhan and Varma, 2009; Dudley, 2012).This effect was described as enhanced permeability and retention (EPR) effect. That can obviously improve the drug effect, and simultaneously reduce the toxicity of drugs and lessen the damage to the body. Since it was discovered that liposomes could discriminately impede the diffusion of ions, many fields including pharmaceutical science, pharmacology, and cell biology has studied the organized assemblies of phospholipids.

RNA interference (RNAi) technology is an important approach to research the pathogenesis and therapeutic method of disease. Due to the utilization of RNAi technology could specifically eliminate or close the expression of specific genes, it has been widely used to explore gene function and gene therapy of infectious diseases and malignant tumors. Numerous researches in many aspects have been already carried on and a series of problems have been solved by using the technology. Cai et al. employed RNAi and found that inhabiting the expression of fibroblast activation protein could inhabit tumor growth (Cai et al., 2013). Since synthetic siRNAs were unable to cross biological membranes by passive diffusion because of their polyanionic nature and high molecular weight. Accordingly, siRNAs demanded transmembrane drug delivery technologies to access the cytoplasm of target cells in generally (Whitehead et al., 2009). The new therapeutics had its own unique mechanism of action and could specifically inhibit previously “undruggable” disease-causing proteins. That had promoted the study of intracellular delivery technologies that were suitable for parenteral administration (Jayaraman et al., 2012). Aurora kinases have three isoforms in mammalian cells: Aurora kinase A (Aurora A), Aurora kinase B (Aurora B) and Aurora kinase C (Aurora C) (Li et al., 2010). Aurora B is a chromosome passenger protein, localized on the centromeres from prophase through the metaphase-anaphase transition. Over expression of Aurora B may be associated with the tumor development. Our laboratory has confirmed that prostate cancer cells would apoptosis by inhibition of Aurora B, which provided a novel method for the treatment of prostate cancer. So the homemade liposomes were used as carriers to entrap the pGUP6-shAurora B or other control substances, and then the liposomes-complex was transfected into PC3 cells or tumor xenograft model mice to observe the transfection efficiency of the liposomes and the tumor growth.

2. Materials and methods

2.1. Materials

DOPE (1,2-dioleoyl-snglycero-3-phosphoethanolamine) and DOTAP (1,2-dioleoyl-3-trimethylammonium) were purchased from Sigma Aldrich. Neutral helper lipid DOPE plays a prominent role to form cationic liposomes. The appropriate addition of DOPE has been identified to increase the gene delivery efficiency of cationic liposomes (Wan et al., 2016).

2.2. Cell lines

Human prostatic carcinoma cell lines, PC3, were obtained from State Key Laboratory of Biotherapy, West China Medical School, Sichuan University. PC3 cells were cultured in RPMI 1640 (Gibico) supplemented with 10% fetal bovine serum and 5% penicillin/streptomycin at 37 °C in a 5% CO_2 humidified atmosphere.

2.3. Plasmid constructions

The pGPU6 vectors harboring caccGGTGATGGAGAATAGCAGTtcaagagACTGCTATTC TCCATCACCttttttg and gatccaaaaaaGGTGATGGAGAATAGCAGTctcttgaACTGCTATTCTCC ATCACC at its *Bbs*I/*Bam*HI sites were prepared for expressing shRNA, specific for interfering with expressions of Aurora B (pGUP6-shAurora B) or asanunrelated sequence control (pGUP6-shNC), respectively. All the constructed plasmids were confirmed by DNA sequencing. Colonies of *E. coli* containing pGPU6 vectors harboring pGUP6-shAurora B or pGUP6-shNC were cultured in Luria Bertani broth containing 50 μg/ml kanamycin. Large-scale plasmid DNA was purified with an Endofree™ Plasmid Giga kit (Qiagen, Hilden, Germany). DNA was eventually dissolved in sterile endotoxin-free water after endotoxin detection and then stored at −20 °C before using.

2.4. Preparation of cationic liposomes

Cationic liposomes were prepared by using an extrusion method for plasmid transfection and animal treatment experiments. Briefly, DOTAP and DOPE with PEG-PE were mixed at a molar ratio of 1:1:0.01 and dissolved in chloroform methanol solution (3:1, volume ratio). The mixture was evaporated at 40 °C in a rotary evaporator to remove the organic solvents and dried in vacuum for 2 h. The dried lipid subsequently dissolved in distilled water was sonicated in ice bath to form liposomes. The particle size was monitored on a Malvern Nano-ZS 90 laser particle size analyzer.

2.5. Transfection efficiency of the homemade cationic liposomes in vitro

Twenty-four hours before transfection, the cells were treated with trypsin and inoculated on the plates. DNA (pGPU6-shAurora B or pGPU6-shNC)/liposomes complexes were prepared at room temperature, maintained in RPMI1640 with free serum for 30 min. In addition, liposomes or medium alone were also used as control. PC3 cells were incubated with the above regents for 4 h, washed three times with PBS and then added to RPMI 1640 supplemented with 10% FBS, with a continued incubation for another 48 h. The transfection efficiency was evaluated by fluorescence microscope.

2.6. Detecting the transfection efficiency of the liposomes in vivo

All animal procedures were approved by the Institutional Animal Care and Treatment Committee of Sichuan University. Tumor xenograft model was established by injecting 1×10^7 PC3 cells into the skin of the back of female athymic nude mice (BALB/c, 6–8 weeks of age, nonfertile and 18–20 g each). When tumors were palpable, mice (N = 7) were randomly distributed into the following four groups and injected intravenously through the tail vein: (i) treatment with 5% glucose solution; (ii) treatment with liposomes; (iii) treatment with pGPU6/shNC; (iv) treatment with pGPU6-shAurora B complexes. The mice were injected intravenously through the vein with 25 μg pGPU6-shAurora B for each mouse, the control groups were injected with 5% glucose, liposomes alone or 25 μg pGPU6-NC.The i.v. route for shRNA delivery was selected in 200 μL volume with a regimen of one time per three days for 8 times. Animals were sacrificed one week after the last treatment, and tumor tissue was fixed in 10% neutral-buffered formalin and embedded in paraffin.

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