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Therapeutic efficacy evaluation of ^{111}in-VNB-liposome on human colorectal adenocarcinoma HT-29/*luc* mouse xenografts

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Abstract

The purpose of this study is to evaluate the therapeutic efficacy of the liposome encaged with vinorelbine (VNB) and ^{111}In-oxine on human colorectal adenocarcinoma (HT-29) using HT-29/*luc* mouse xenografts. HT-29 cells stably transfected with plasmid vectors containing luciferase gene (*luc*) were transplanted subcutaneously into the male NOD/SCID mice. Biodistribution of the drug was performed when tumor size reached 500–600 mm^3. The uptakes of ^{111}In-VNB-liposome in tumor and normal tissues/organs at various time points postinjection were assayed. Multimodalities, including gamma scintigraphy, bioluminescence imaging (BLI) and whole-body autoradiography (WBAR), were applied for evaluating the therapeutic efficacy when tumor size was about 100 mm^3. The tumor/blood ratios of ^{111}In-VNB-liposome were 0.044, 0.058, 2.690, 20.628 and 24.327, respectively, at 1, 4, 24, 48 and 72 h postinjection. Gamma scinitigraphy showed that the tumor/muscle ratios were 2.04, 2.25 and 4.39, respectively, at 0, 5 and 10 mg/kg VNB. BLI showed that significant tumor control was achieved in the group of 10 mg/kg VNB (^{111}In-VNB-liposome). WBAR also confirmed this result. In this study, we have demonstrated a non-invasive imaging technique with a luciferase reporter gene and BLI for evaluation of tumor treatment efficacy in vivo. The SCID mice bearing HT-29/*luc* xenografts treated with ^{111}In-VNB-liposome were shown with tumor reduction by this technique.

Keywords: ^{111}In-VNB-liposome; Human colorectal adenocarcinoma; HT-29/*luc* bioluminescence imaging; Whole-body autoradiography

1. Introduction

The vinca alkaloids, such as vincristine, vinblastine and vinorelbine (5′-nor-anhydrovinblastine, VNB), represent one of the most widely used classes of antineoplastic agents. The mechanism of their cytotoxicity against the cancer is through the inhibition of microtubule dynamics and assembly at the metaphase/anaphase transition, leading to apoptosis and cell growth arrest during metaphase [1,2]. VNB differs from other vincas with a modification of the catharanthine moiety, and exhibits a broader spectrum of anticancer activity but reduced neurotoxicity [2,3]. Since vinca alkaloids inhibit cell growth exclusively during metaphase, it is advantageous to have tumors exposed to the drug for a longer time so that more tumor cells can experience the sensitive part of the cell cycle. One way to approach this is to encage the drug in a slow-release liposomal system. This can result in the liposomal formulations of VNB that exhibit enhanced therapeutic efficacy as compared to the free drug.

Reporter genes, on the other hand, provide an elegant alternative strategy for molecular and genetic studies that is easier, less expensive, more accurate and quantifiable than the standard hybridization techniques [4]. Protein or enzyme translated from the reporter gene, which is not normally

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found in the eukaryotic cells, can be detected by assaying their substrates or metabolites. Real-time imaging of these products could be accomplished by digital imaging microscopy, highly sensitive photon counting facilities, such as the charge coupled device, optical imaging or non-invasive radiological devices, such as magnetic resonance imaging (MRI), single photon emission computerized tomography (SPECT) and positron emission tomography (PET). Luciferase gene (*luc*) from firefly is one of the most widely used reporter genes for bioluminescence imaging (BLI). Animals that carry *luc* gene offer cell populations with sufficient stability of the reporter gene for various studies [5]. BLI could be used for tracing tumor growth and development in vivo, drug treatment efficacy as well as a broad dynamic range of quantification with excellent resolution [6–9].

Liposome, usually 50–500 nm in diameter, is a small artificial spherical vesicle that can be produced from natural non-toxic phospholipids and cholesterol. With both hydrophobic and hydrophilic characteristics, liposomal carrier turns out to be a very promising drug delivery system to increase the efficacy of the drug while decreasing its toxicity due to the following reasons: (a) increase the drug stability in vivo; (b) reduce the unwanted toxicity due to drug actions; (c) improve the drug bioavailability and (d) provide the targeted drug delivery or sustained the drug release near the site of action [10]. However, pharmacokinetics of a drug may be affected by its association with the liposome either entrapped in the internal space or bound to its bilayers. Liposomal drug delivery system has been shown with many benefits, such as increasing therapeutic efficacy for anticancer and antimicrobial therapies, immuno-, inflammation and gene therapies, and diagnostic applications [11]. Liposome could be rapidly cleared up by the reticuloendothelial system (RES) and resulted in higher accumulation in the spleen [12,13]. The unpredictable patterns of extravasation and lack of long-term physicochemical stability are two other two disadvantages [14]. Liposomes with modified lipids or inserted derivative polyethyleneglycol (PEG) will significantly increase its retention in the bloodstream and accumulation in tumors [15–17]. The development of pegylated, so-called stealth, liposomes in the last decade has rekindled the interest for clinical application in cancer treatment and even conjugation with radiopharmaceuticals, such as Ga-67, In-111 or Tc-99m, to image tumors in vivo for diagnostic or therapeutic improvement in a series of preclinical studies [18–22]. In this study, we developed a liposomal delivery system, combined VNB and ^{111}In-oxine to evaluate its diagnostic and therapeutic efficacy in HT-29/*luc*-bearing mice with multimodalities of molecular imaging techniques.

2. Materials and methods

2.1. Cell culture

The HT-29 colorectal carcinoma cell line was purchased from the Bioresource Collection and Research Center, Hsinchu, Taiwan. The cell line was cultured in RPMI-1640 with 10% fetal bovine serum (Hyclone, Utah, USA) and supplemented with L-glutamine, sodium biocarbonate and 100 μg/ml penicillin–streptomycin. The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Stably transfected HT-29/*luc* cells were grown in the same condition but contained 500 μg/ml G418 (Merck, US) to maintain stable expression of *luc* gene.

2.2. Plasmid construction and transfection

The *luc* gene was sliced from pGL3-basic with *Nhe*I and *Xba*I. The EGFP of the pEGFP-C1 was sliced to form pC1 with *Nhe*I, and *Hind*III. The *luc* gene was subcloned into pC1 with Klenow and ligase to form the final plasmid vector as shown in Fig. 1.

The day before transfection, 5×10^5 cells were seeded in a 60 mm ψ dish containing 5 ml culture medium. The cells were transfected with constructed vectors and maintained in the incubator until the cell number reached 40%–60% of confluence. Since the transfection efficiency is very sensitive and can be affected by culture condition, it is important to follow a standard seeding protocol for each experiment.

For the transfection dish, both 10 μg pC1-*luc* plasmid and 10−μl jetPEI reagent (Polypuls-transfection, Illkirch, France) were diluted into 250 μl NaCl solution (150 mM), respectively. Two solutions were mixed and incubated at room temperature for 30 min to allow the formation of DNA-cationic complex. The mixture was added onto HT-29 cells, and incubated for 24 h, then subcultured into the selective medium. Transfected HT-29/*luc* cells were selected with 500 μg/ml G418. The stably transfected cells were selected by G418 for two weeks. The surviving colonies were screened for *luc* gene activity by BLI in vitro using the Wallac 1420 Multilabel Counters (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). The HT-29/*luc* clone was characterized for stable luminescence expression in vitro and tumorigenesis in vivo.

2.3. Preparation of triethylammonium salts of sucrose octasulfate

The aqueous solution of triethylammmonium sucrose octasulfate (TEA-SOS, 0.6 M triethylammonium, pH 5.7–6.2) was prepared according to the original proprietary method of Hermes Biosciences Inc. TEA-SOS was prepared from the sodium salt of sucrose octasulfate using ion exchange chromatography. In brief, 6 g of sucrose octasulfate (sodium salt) was dissolved in 16.57 ml of water to give a final concentration of 0.313 M. A Dowex 50W-8X-200 cation exchange resin was employed to prepare the acidic form of sucrose octasulfate. Defined resin was washed twice with 2 vol of 1 N NaOH, then with ddH_2O to neutralize pH, washed twice with 2 vol of 1 N HCl, and finally washed with ddH_2O to neutralize again. This step was repeated once more. After the column was poured, it