PIPELONGUMINE-ELUTING NANOFIBER MATS FOR APPLICATION OF DRUG-ELUTING STENT AND ANTICANCER ACTIVITY AGAINST CHOLANGIOCARCINOMA

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Abstract - Piperlongumine-eluting nanober was fabricated using poly(DL-lactide-co-glycolide) copolymer by electrospinning method for application in gastrointestinal (GI) stent to treat cholangiocarcinoma cells. Piperlongumine showed sustained release over one month in vitro. Piperlongumine eluted from nanofiber was similarly inhibited growth of cancer cells and induced apoptosis against HuCC-T1 human cholangiocarcinoma cells in vitro.Furthermroe, piperlongumine-eluting nanofiber coated GI stent showed efficiently inhibition of tumor growth of HuCC-T1 tumor at tumor-xenograft study. We suggest piperlongumine-eluting nanofiber for GI stent as promising medical equipment.

Keywords - Piperlongumine, nanofiber, drug eluting stent, cholangiocarcinoma

I. INTRODUCTION

Cholangiocarcinama (CCA) is an epithelial cancer lining in bile duct area and is known as an aggressive cancer [1]. CCA is mostly diagnosed as an advanced stage and then this makes it difficult to cure [2]. Especially, CCA has very low response rate against conventional therapeutic regimen such as chemotherapy and radiotherapy since advanced state of CCA is frequently impossible to remove by surgical treatment [1-3]. GI stent for CCA is normally used to prolong patient life time. However, tumor ingrowth into the stent mostly induces obstruction problem because bare metal stent or polymer membrane-covered stent only support mechanical passage and has no anticancer activity. From these points of view, drug-eluting stent (DES) has been extensively investigated to treat CCA, i.e. anticancer –agent incorporated biodegradable membrane was coated onto GI stent and then this membrane elutes anticancer agent for a long time to retain biliary drainage [4,5]. Drug-eluting stent is still regarded as a promising option to treat CCA.Biodegradable nanofiber mats have been extensively investigated for application in biomedical field [6-8]. Since they have similar scale/environment compared to microenvironment of organs or tissues of human, nanofiber mats are regarded as an appropriate device for sustained drug delivery in the medical devices [7,8]. Furthermore, nanofiber is superior devices for controlling drug release behavior or drug absorption into tissues since their nano-scale structure enable us to control them easily.In this study, we fabricated drug-eluting nanofiber coated GI stent for treatment of CCA. Piperlongumine was used for drug-eluting nanofiber coated stent. Piperlongumine is known to produce reactive oxygen species (ROS) and then has medicinal activity against cancer cells [9]. They induce apoptosis of cancer cellsin various cancer cell types and has minimum toxicity against normal cells or tissues [9,10]. Furthermore, piperlongumine has an antitumor activity against tumor xenograft model and no apparent toxicity in normal mice [11].

II. DETAILS EXPERIMENTAL

2.1. Materials

Piperlongumine was purchased from Wako Pure Chem, Co. Ltd., (Osaka, Japan). Poly(DL-lactide-co-glycolide) (PLGA 503H) was purchased from Evonik Ind., (Darmstadt, Germany). Chloroform, acetone and dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich Chem Co. (St. Louis, MO, USA). GI stent (Silicon-membrane covered stent) was purchased from M.I. Tech. (Pyeongtaek-si, Korea). RPMI1640 media and fatal bovine serum (FBS) was purchased from Life Tech. Inc. (Grand Island, NY, USA). All reagent and organic solvents was used as HPLC-grade.

2.2. Fabrication of piperlongumine-incorporated PLGA nanofiber mates and covered with stent

Piperlongumine (100mg) and PLGA (900mg) was dissolved in 15ml chloroform or acetone and then spray-dried onto aluminium foil of rolling collector using electro spray coating equipment (ES-Biocoater, NanoNC, Seoul, South Korea). Organic solvent containing polymer/drug was introduced into syringe (24G) and then sprayed onto a rolling collector (10kV, 50□l/min).

2.3. Characterization of piperlongumine-incorporated PLGA nanofiber

Morphology of piperlongumine-incorporated nanofiber was observed with field emission electron scanning microscope (S-4800, Hitachi, Japan)

Drug release study was carried out in vitro using phosphate-buffered saline (PBS, 0.01M, pH7.4). Naofiber mats incorporating piperlongumine (20mg) was cutted and introduced into 50ml falcon tube with 40 ml PBS. Released piperlongumine was measured with UV-spectrophotometer (UV-1601, Shimadzu Co. Ltd., Osaka, Japan) at 325 nm.

2.4. Cell culture and anticancer activity of piperlongumine-incorporated PLGA nanofiber

HuCC-T1 human CCA cells were maintained with RPMI1640 medium supplemented with 10% FBS and 1% antibiotics.

Anticancer activity: Prior to do cell culture study, piperlongumine was eluted from nanofiber mats as described in drug release study. 1×10^4 cells were seeded into 96 wells and then eluted piperlongumine was treated to cells for 2 day. After that, cell viability was measured with MTT cell proliferation assay. 30 · l of MTT solution (5mg/ml PBS) was added to cells. 4h later, supernatants was removed and then 100 · l of DMSO was added. Viability of cells were measured with microplate reader (Infinite M200 Pro, Tecan, Mannedorf, Switzerland) at 570nm.

2.5. Animal tumor xenograft study

SNU478 human CCA cells (1×10^6 cells) were implanted into the back of BALB/c nude mice (20 g, 5week, male). When tumor size was reached to larger than 4 mm, piperlongumine-incorporated nanofiber mats were implanted (Piperlongumine dose: 10 mg/kg). PBS was subcutaneously administered for control. Piperlongumine dissolved in Cremophor® EL/ethanol solution (1/1) was diluted with PBS ten times and subcutaneously injected for piperlongumine treatment.4 weeks later, tumor volume was measured using Vernier Calipers.All animal study was strictly kept the guidelines of Animal Treatment and Research Council of Pusan National University.

III. RESULTS AND DISCUSSION

3.1. Characterization of piperlongumine-incorporated nanofiber coated stent

Figure 1 shows the piperlongumine-incorporated nanofiber mats. As shown in Figure 1, piperlongumine-incorporated nanofiber mats have fibrous structure with small diameter less than 100~500nm. Empty nanofiber mats also showed almost similar structures compared to piperlongumine-incorporated nanofiber (data not shown).Piperlongumine-incorporated nanofiber mats showed some granules. These results indicated that piperlongumine-incorporated nanofiber was successfully prepared.

Figure 2 the piperlongumine release from PLGA nanoiber mats. As shown in Figure 2, piperlongumine was continuously over one month. Burst release was shown for 2 days at piperlongumine release from PLGA nanofibers.

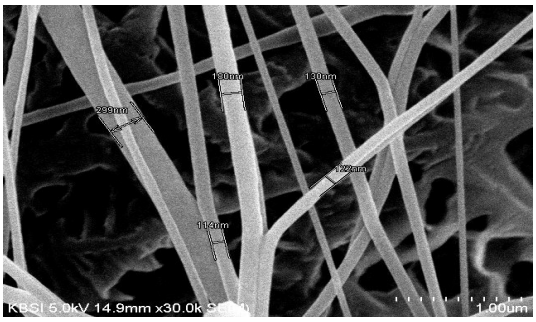


Figure 1.Pipelongumine-incoeporatedPLGA nanofiber mats.

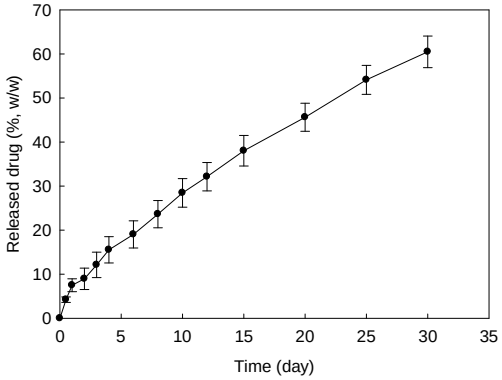


Figure 2.Piperlongumine release from PLGA nanofibers.

3.2. Anticancer activity of pipelongumine against cholangiocarcinoma cells

Antiancer activity of piperlongumine and piperongumine-incorporated nanofibers were assessed with HuCC-T1 and SNU478 human cholangiocarcinoma cells as shown in Figure 3.Piperlongumine or piperlongumine-incorporated nanofiber was dissolved in DMSO was and then treated to cells. To test anticancer activity of released piperlongumine, piperlongumine released at 2 days was also harvested and then treated to cells. As shown Figure 3, piperlongumine dose-dependently inhibited viability of HuCC-T1 cells and SNU478 human cholangiocarcinoma cells.Piperlongumine-incorporated nanofiber also showed almost similar anticancer activity compared to piperlongumine itself. Furthermore, anticancer activity of releasedpiperlongumine was not significantly changed compared to piperlongumine or piperlongumine-incorporated nanofiber. These results indicated that anticancer activity of piperlongumine was not signicantly changed during nanofiber fabrication process and drug release study.

Anticancer activity of piperlongumine-incorporated nanofibers was also assessed with animal tumo model of SNU478-bearing mice as shown in Figure 4. Interestingly, piperlongunmine-incorporated nanofiber was efficiently inhibited growth of SNU 478 human cholangiocarcinomacells in vivo as shown in Figure 4. Piperlonguminenanofibers showed higher

efficacy than piperlongumine injection. These results might be due to the sustained release property of nanofiber mats.

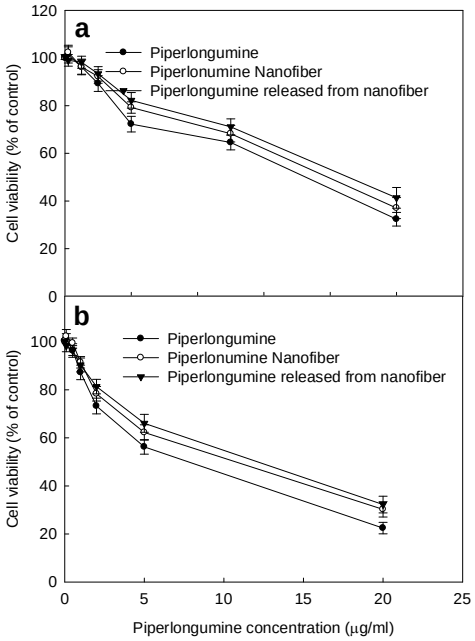


Figure 3.Anticancer activity of piperlongumine and piperlonguminenanofiber. For piperlongumine released from nanofiber, piperlongumine was released from nanofiber for 2 days and then piperlongumine concentration was measured following treatment of cells.

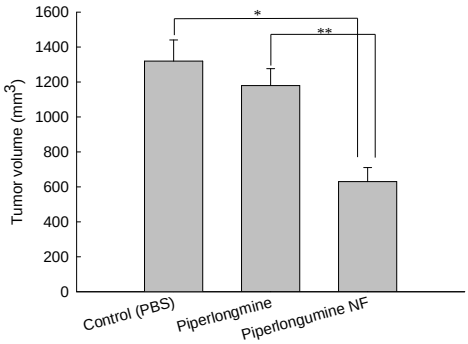


Figure 4.Anticancer activity of piperlongumine-incorporated nanofiber.Piperlongminedose : 10 mg/kg). *, **: p< 0.01. Tumor volume was average ± S.E. from 4 mouse. NF = nanofiber.

CONCLUSIONS

Piperlongumine-incorporated nanofiber mats was fabricated using biodegradable PLGA copolymer for application of DES. Piperlongumine was released continuously over one month and they have similar anticancer activity against human cholangiocarcinoma cells in vitro. Furthermore, they showed higher anticancer activity at in vivo tumor xenograft model of SNU478 human cholangiocarcinoma cells.

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