In vivo antitumor effect of cromolyn in PEGylated liposomes for pancreatic cancer

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A B S T R A C T

A PEGylated liposomal formulation of cromolyn, composed of dipalmitylophosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phospho-ethanolamine-N-[methoxy(polyethyleneglycol)-2000] (DSPE-mPEG2000), has been developed with the purpose of improving the antitumor activity of cromolyn for human pancreatic adenocarcinoma. In stability study, the amount of proteins adsorbed onto the PEGylated liposomes encapsulating cromolyn was 4.5-fold lower than the non-PEGylated liposome. In vitro study showed that the cromolyn in PEGylated liposome exhibited better anti-proliferative effect in BxPC-3 cells than in Panc-1 cells, which indicates higher level of endogenous S100P protein in BxPC-3 cells than in Panc-1 cells as a target protein for this drug. Moreover, the combination of cromolyn with gemcitabine in PEGylated liposomes demonstrated the strongest cytotoxicity to BxPC-3 pancreatic cancer cells in vitro and the highest anti-tumor activity against the BxPC-3 tumor bearing nude mice in vivo. Thus, this PEGylated liposomal formulation of cromolyn is expected to provide a novel approach to the treatment of pancreatic cancer in the future.

1. Introduction

Pancreatic cancer is the fourth most common cause of adult cancer death, accounting for an estimated 42,470 new cases and 35,240 deaths in USA in 2009. The high mortality rate is due to the high incidence of metastatic disease at initial diagnosis, the aggressive clinical course and the lack of adequate systemic therapies. Therefore, the overall 5-year survival rate is no more than 5% [1–3]. Radical surgery is still the only procedure that can completely eradicate this disease, and gemcitabine has been a global reference regimen for pancreatic cancer because of its favorable toxicity profile and modest ability to palliate typical pancreatic cancer symptoms. However, surgery and/or gemcitabine do not prolong the survival rate remarkably, so other drugs have been given in combination with gemcitabine such as 5-fluorouracil, oxaplatin, capecitabine and erlotinib [4,5]. Since the therapeutic efficacy of these gemcitabine combinations used in clinics is not enough to dramatically increase the therapeutic efficacy of pancreatic cancer, new targeting strategies and therapeutic approaches are needed to improve the survival rate and quality of life for pancreatic cancer patients.

S100P is a member of the S100 family of EF hand Ca^{2+} binding proteins and have been implicated in the regulation of a variety of intracellular and extracellular processes, including cell cycle, cell growth, differentiation and metabolism [6]. Overexpression of S100P has been observed in various cancers such as breast cancer, lung cancer, colon cancer and pancreatic cancer, with the extent of the overexpression being positively correlated with the degree of malignancy. Furthermore, it is recently reported that S100P is able to bind to the receptor for advanced glycation end-products (RAGE) which is associated with many inflammation-related pathological states such as vascular disease, cancer, neurodegeneration and diabetes [7]. Activation of RAGE is known to stimulate extracellular signal-regulated kinase (ErK) and nuclear factor-κB (NF-κB) activity and to increase the cell invasion and growth in pancreatic cancer cells [8].

Originally, cromolyn was developed as an anti-inflammatory drug used for prophylactic treatment of bronchial asthma and allergic rhinitis and it is currently administered as an intranasal solution, powder or inhalation [9,10]. Regarding the mechanism and involvement of S100 proteins in pancreatic cancer, cromolyn has previously been shown to bind specifically to other members of the S100 protein family (S100A1, S100A12, S100A13) [11,12]. In addition, cromolyn has recently been found that it binds S100P, blocks the interaction with RAGE, and inhibits tumor growth [13], which provides useful information on the use of cromolyn for cancer therapy. However, the way to deliver cromolyn into the body is very limited due to its physico-chemical properties.

It is well known that liposomes made from naturally occurring phospholipids are biocompatible carriers, and their application to drug delivery systems is known to reduce the drug toxicity and increase the therapeutic efficacy [14]. Although rapid clearance of the conventional liposomes by the reticular endothelial system
2. Materials and Methods

2.1. Materials

Dipalmitylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), cromolyn sodium salt (disodium 1,3-bis [(2-carboxylatychromon-5-y)i]oxy]-2-hydroxy-propane), 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyterrazolium bromide (MTT), dimethylsulfoxide (DMSO), chloroform (CHCl₃), trypan blue, crystal violet and Sephadex® G-75 were purchased from Sigma Chemical Co. (St. Louis, MO). 1,2-Distearoylsn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG2000) was purchased from the Avanti Polar Lipids (Alabaster, AL). Gemicitabine HCl, commercially known as Gemzar®, was purchased from Shinwon Pharmacy Co. (Seoul, Korea). All reagents and solvents were of analytical grade or better.

2.2. Preparation of PEGylated liposomes

PEGylated liposomes, composed of DPPC : DMPC : DSPC : DSPE-mPEG2000 (4:1:1:0.1−1.0 molar ratio), were prepared using a reverse-phase evaporation vesicle (REV) method. Briefly, DPPC, DMPC, DSPC and DSPE-mPEG2000 were dissolved in 1 mL of chloroform. After evaporation of solvent at room temperature, the formed lipid film was suspended in 1 mL of freshly hydrated diethyl ether, to which cromolyn in 1 mL of phosphate buffered saline (PBS, pH 7.4) was added. The mixture was sonicated using a bath type sonicator (Laboratory Supplies Co., Inc., NY) for 3 min until the mixture became a homogeneous dispersion and the organic solvent was then removed by a rotary evaporator (Laborota 4000, Heidolph, Italy) at 41 °C. Liposomes were downsized by extrusion through 0.45 μm and 0.2 μm polycarbonate membrane filters 10 to 20 times using a Lipex™ extrusion device (Avestin Inc. Toronto, Canada). The unencapsulated drug was removed from the liposome suspension by gel chromatography on a 1×12 cm Sephadex® G-75 column eluted with PBS.

2.3. Characterization of liposomes

The encapsulation efficiency of cromolyn in liposomes was evaluated by the Bligh and Dyer extraction method. Briefly, 100 μL of liposomes was mixed with 150 μL of PBS, 250 μL of methanol and 1 mL of chloroform in a glass tube to form a homogenous solution. After centrifugation, the mixture was separated into two clean layers. The bottom layer contained phospholipids and other hydrophobic materials, and the top layer contained cromolyn and other hydrophilic materials. The organic phase (bottom layer) was removed and chloroform was added again. The mixture was centrifuged, and these procedures were repeated three times. The amount of cromolyn in the supernatant was determined spectrophotometrically at 326 nm using an UltraSpec 2000 UV−VIS spectrophotometer (Pharmacia Biotech, Cambridge, UK).

The size distribution and zeta potential of liposomes were determined by a dynamic laser-light scattering system (NICOMP 380ZLS, Inc., Santa Barbara, CA) using He-Ne laser light source. Measurements were carried out at room temperature with a 90 degree detection angle.

2.4. Stability assay

The in vitro stability of liposome in the presence of serum proteins was determined by measuring the amount of proteins adsorbed onto the liposomes. One percent (w/v) of bovine serum albumin was dissolved in PBS (pH 7.4) and mixed with liposomal solution of each formulation at 37 °C. After incubation for 0.5, 3, 12, 24 and 48 h, samples were centrifuged at 13000×g for 15 min. The free serum albumin in the supernatant was removed and PBS was added, and these procedures were repeated three times. The amount of proteins adsorbed onto the liposomes was measured using a BCA Protein Assay Kit (Pierce, Rockford, IL) with bovine serum albumin as a standard. Twenty-five μL of samples were put into 96 well plates and mixed with 200 μL of BCA working reagent. The absorbance was measured spectrophotometrically at 570 nm using 1420 Multilabel Counter (Victor3, PerkinElmer Life and Analytic Sciences, USA).

2.5. MTT assay

The viability of Panc-1 and BxPC-3 cells treated with cromolyn was assessed by MTT assay. Briefly, Panc-1 (5×10⁵/well) and BxPC-3 cells (6×10⁵/well) were seeded in 96-well plates and incubated overnight at 37 °C. Culture medium was removed and replaced with fresh medium containing PBS, empty liposome, 300 μM free cromolyn (cro), 300 μM PEGylated liposomal cromolyn (PEG-lipo-cro), 30 μM PEGylated liposomal gemicitabine (PEG-lipo-gem), or a combination of 300 μM PEG-lipo-cro plus 30 μM PEG-lipo-gem. After 48 h incubation at 37 °C, 10 μL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 h. The supernatant was removed, and then the formed formazan salts were dissolved by 100 μL of DMSO in shaking plates for 30 min. Absorbance was measured at 570 nm using an ELISA reader (EL 800, BIO-TEK, USA). The percent growth (% Growth) was calculated according to the following equation:

\[
\%\text{Growth} = \frac{OD_{570}(\text{sample})}{OD_{570}(\text{control})} \times 100
\]

2.6. In vivo animal study

Five-week-old female BALB/c-nu nude mice (from Japan SLC, Inc., Japan) were maintained in appropriately isolated cages with free access to drinking tap water and food on a daily 12-hour light/dark cycle. The BxPC-3 cells, which had been grown in RPMI 1640 with 10% FBS, were collected and resuspended in PBS. A total of 1.5×10⁷ cells in 70 μL of PBS mixed with 50 μL of Matrigel Matrix (BD Bioscience, Bedford, USA) were inoculated subcutaneously on the right flank of a nude mouse and the tumors were allowed to grow (Day 0).

One week after cell injection when the tumor volume has reached about 140 mm³ (Day 7), the mice were randomly divided into 6 groups, each group having 6 mice (n=6) except for the control group (n=4). Each mouse was treated with PBS (control), empty liposome, cromolyn (10 mg/kg), non-PEGylated liposomal cromolyn (10 mg/kg), PEG-lipo-cro (10 mg/kg), PEG-lipo-gem (80 mg/kg) or the combination of PEG-lipo-cro (10 mg/kg) and PEG-lipo-gem (80 mg/kg). Six different formulations were injected into the mice intravenously twice a week for 4 weeks, a total of 8 injections. The tumor size was measured twice a week with Vernier caliper (Mitutoyo Co., Japan) across its two perpendicular diameters, and the volume was estimated using the following equation:

\[
\text{Tumor Volume} = 0.5 \times (W^2 \times L)
\]
where W is the smaller perpendicular diameter and L is the larger perpendicular diameter. Tumors were excised at the end of the in vivo experiment (Day 42) and embedded in OCT compound (Tissue-Tek®), and cryo-sectioned into a 7 μm length using a Leica CM3050S cryostat (Leica Microsystems, Wetzlar, Germany) for histological analysis. All animal experiments were approved by the SMU-IACUC of the Sookmyung Women’s University, Korea.

2.7. Immunohistochemistry

Detection of apoptosis in tumor cells was performed using In Situ Cell Detection Kit (Roche Diagnostics, Mannheim, Germany), which is based on a terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) method and the DNA strand breaks are detected by enzymatic labeling of free 3’-OH ends with modified nucleotides. The tissue sections were fixed with acetone : ethanol (30% : 70%) solution at room temperature and rinsed with PBS. Then the sections were incubated in 100 μL of 3% hydrogen peroxide, and permeabilized with 100 μL of 0.1% Triton X-100 for 5 min. Sections were rinsed again and developed with the TUNEL reaction mixture in a humidified atmosphere in the dark. The sections were rinsed and visualized using Converter-POD with diaminobenzidine (DakoCytomation, Glostrup, Denmark) and then counterstained with Harris’ hematoxylin. The slides were air-dried at room temperature, and cover slips were mounted using Permount. The TUNEL-positive cells were analyzed under the Olympus IX71 microscope (Olympus America, Melville, NY) at 400× magnification.

2.8. Statistical analysis

Results were expressed as a mean±standard deviation (SD). Statistical analysis was performed using an unpaired t test (between two groups) or one way analysis of variance (ANOVA) (among three or more groups) with computer software SPSS 12.0. A P-value<0.05 or P<0.01 was considered statistically significant.

3. Results

3.1. Physical properties of PEG-lipo-cro

Physical properties of the cromolyn-encapsulatedPEGylated liposomes (or PEG-lipo-cro), composed of DPPC: DMPC: DSPC: DSPE-mPEG2000 were evaluated by measuring particle size, zeta potential and encapsulation efficiency after the addition of different molar ratio of DSPE-mPEG2000 (Table 1). After extrusion through 0.2 μm polycarbonate membrane filters, liposome showed an average diameter of 146–188 nm with highly homogenous distribution. The amount of DSPE-PEG2000 does not affect the size of liposomes until the molar ratio of DSPE-mPEG2000 reached 0.3. However, when the ratio increased up to 1, the total amount of lipid increased due to increase of the amount of DSPE lipid, which affected the increase of particle size. The zeta potential of PEGylated liposomes was more negative in proportion to an increase of the amount of DSPE-mPEG2000 in liposomes. The drug encapsulation efficiency of liposomes of various ratios of DSPE-mPEG2000 at 0.1, 0.2, 0.3, 0.5 and 1 was 28%, 26%, 23%, 14% and 7%, respectively. When the molar ratio of DSPE-mPEG2000 was increased from 0.1 to 1, the encapsulation efficiency of cromolyn into the liposome dropped from 28% to 7.7%. During the storage at 4 °C up to 14 days, drug precipitation or liposome aggregation did not occur (data not shown). Therefore, the best ratio of liposome formulation was found to be DPPC: DMPC: DSPC: DSPE-mPEG2000 = 4: 1: 1: 0.1, and this formulation was used for further experiments.

2.9. Measurement of protein adsorption onto PEGylated liposomes

The protein adsorption onto the liposomes causes aggregation and instability of liposomes in the presence of serum proteins. Thus, the amount of protein adsorbed on the surface of liposomes was measured by BCA protein assay. Fig. 1 shows that the amount of protein adsorbed on the non-PEGylated liposome increased during the 48 h incubation period, whereas PEGylated liposomes encapsulating cromolyn or gemcitabine showed only a small amount of protein adsorption. The protein adsorption on PEGylated liposomes (empty, cromolyn or gemcitabine encapsulated) was 4.5-fold lower than the non-PEGylated liposome in 48 h (P<0.01). Significant amount of protein adsorption or aggregation did not occur when the PEG-lipo-cro

Table 1

<table>
<thead>
<tr>
<th>Cromolyn-Encapsulated PEGylated Liposomes</th>
<th>Encapsulation efficiency (%)</th>
<th>Mean diameter (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar ratio*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:1:1:0.1</td>
<td>28</td>
<td>158 ± 4</td>
<td>−2.23</td>
</tr>
<tr>
<td>4:1:1:0.2</td>
<td>26</td>
<td>146 ± 8</td>
<td>−3.14</td>
</tr>
<tr>
<td>4:1:1:0.3</td>
<td>23</td>
<td>166 ± 4</td>
<td>−7.54</td>
</tr>
<tr>
<td>4:1:1:0.5</td>
<td>14</td>
<td>181 ± 5</td>
<td>−6.37</td>
</tr>
<tr>
<td>4:1:1:1.0</td>
<td>7</td>
<td>188 ± 3</td>
<td>−12.34</td>
</tr>
</tbody>
</table>


3.2. In vitro stability assay of PEGylated liposomes

Fig. 2. In vitro anti-proliferative effect of PEG-lipo-cro in Panc-1 and BxPC-3 cells (P<0.01 against PBS control).
was mixed with the PEG-lipo-gem, presumably due to the fact that PEG coats the surface of liposomes by making steric barriers to the water, resulting in repulsive interaction between PEG and serum proteins.

3.3. Inhibition of proliferation in Panc-1 and BxPC-3 Cells

To investigate the anti-proliferative effect of cromolyn in various formulations in pancreatic cancer cells, Panc-1 and BxPC-3 cells were treated with PBS, empty liposome, free cromolyn, PEG-lipo-cro, PEG-lipo-gem, or combination of PEG-lipo-cro with PEG-lipo-gem for 48 h. As shown in Fig. 2, anti-proliferative effect of cromolyn in PEGylated liposomes was better than the free drug, and this effect was more enhanced in BxPC-3 cells than Panc-1 cells as the BxPC-3 cells exerted higher level of endogenous S100P protein than the Panc-1 cells \( (P<0.01) \). Moreover, anti-proliferative effect of the combination of PEG-lipo-cro with PEG-lipo-gem in BxPC-3 cells was 2.6-fold higher than the PEG-lipo-gem alone \( (P<0.01) \), though the enhanced anti-proliferative effect of combination formulation was not seen in Panc-1 cells. This is presumably due to the fact that S100P is a main target for cromolyn (see the Fig. S-1 in Supplementary Pages for the result), and the anti-proliferative effect of cromolyn is enhanced owing to the PEGylated liposome formulation. Also, the anti-proliferative effect of cromolyn is further enhanced when used together with gemcitabine. Neither empty liposome nor PBS control did affect the proliferation of both Panc-1 and BxPC-3 cells \( (P>0.05) \).

Long-term cell viability after the same treatment was evaluated using the clonogenic assay under light microscope and was found to be in accordance with the results shown in Fig. 2. (see the Fig. S-2 in Supplementary Pages for the result), NF-κB activity would be expected to be blocked in the same way as shown in Fig. 2, which was confirmed using the BxPC-3 cells that were transiently transfected with a NF-κB-driven luciferase reporter construction (see the Fig. S-3 in Supplementary Pages for the result).

3.4. In vivo anti-tumor activity of PEG-lipo-cro in xenograft mice

In vivo anti-tumor effect of PEG-lipo-cro was evaluated using a xenograft nude mouse model with human pancreatic cancer cells. One week after the injection of BxPC-3 pancreatic cancer cells in the flank of BALB/c-nu nude mice (Day 7), mice were divided into six treatment groups \( (n=6) \) including the control group \( (n=4) \). Various drug formulations were administered intravenously twice a week for a total of 8 times; control (PBS), empty liposome, free cromolyn 10 mg/kg, PEG-lipo-cro 10 mg/kg, PEG-lipo-gem 80 mg/kg, or PEG-lipo-cro 10 mg/kg with PEG-lipo-gem 80 mg/kg. Tumor volume was measured twice a week until Day 42. No significant weight loss was observed in any of the treatment groups, indicating that all the treatments were well tolerated.

As shown in Fig. 3, tumor volume increased rapidly when the mice were treated with PBS or empty liposome, with little difference between these two groups \( (P>0.05) \). After 42 days, the tumor growth inhibition by PEG-lipo-cro treatment was 2.2-fold higher than the PBS treatment and its antitumor activity being more effective than free cromolyn \( (P<0.01) \). Although the average tumor volume between the PBS or free cromolyn treated mice was statistically different \( (P<0.01) \), the anti-tumor activity of free cromolyn was not strong enough compared to other liposome-mediated cromolyn formulations. This result strongly suggests that the potency of cromolyn as an anticancer drug and the possibility of PEGylated liposome as a suitable delivery system for cromolyn were well proved. In addition, the combination of PEG-lipo-cro 10 mg/kg with PEG-lipo-gem 80 mg/kg was the most effective among the formulations tested in inhibiting tumor growth, showing 3.2-fold higher activity than the control group. This indicates that the combination therapy using PEGylated liposomes encapsulating cromolyn and gemcitabine might prove useful in treating the pancreatic cancer.

Representative photographs of each group's tumor mass at the end of the in vivo experiment (Day 42) were shown in Fig. S-4 (Supplementary Pages). There are sharp differences in tumor size in each treated group, where the tumor volume from the treatment of the combination of PEG-lipo-cro with PEG-lipo-gem \( (f) \) was the smallest among tested, followed by PEG-lipo-gem \( (e) \), PEG-lipo-cro \( (d) \), free cromolyn \( (c) \), empty liposome \( (b) \) and PBS control \( (a) \). This result also indicates that the therapeutic efficacy of the combination of PEG-lipo-cro with PEG-lipo-gem was the best among tested in BxPC-3 xenograft nude mouse model.

3.5. Effect of PEG-lipo-cro on apoptosis

To study the effect of PEG-lipo-cro on the apoptosis of BxPC-3 cells in xenograft nude mice, immunohistochemical staining was done using TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling) assay. Fig. 4 shows representative photos in which apoptosis was induced in a population of cells in the tumors isolated from the mice 24 h after i.v. injection of PBS, empty liposome, free cromolyn, PEG-lipo-cro, PEG-lipo-gem or the combination of

![Fig. 3. Improved therapeutic efficacy of PEGylated liposomal cromolyn (PEG-lipo-cro) in BxPC-3 pancreatic cancer xenograft mouse \( (P<0.01) \).](image)

![Fig. 4. TUNEL assay of tumor tissues after the treatment of the xenograft mice with various treatments.](image)
PEG-lipo-cro with PEG-lipo-gem. Nuclei of TUNEL-positive apoptotic cells were stained brown and their shapes were condensed or segmented, whereas nuclei of non-apoptotic cells were stained violet by hematoxylin. Higher incidents of apoptotic bodies were found in the tumor cells from mice treated with PEG-lipo-cro, PEG-lipo-gem, or combination therapy than the free cromolyn or PBS control.

4. Discussion

It has been reported that S100P is overexpressed in more than 90% of pancreatic cancer due to the endoplasmic reticulum (ER) stress response and hypomethylation of the S100P gene under hypoxic condition in tumors [18,19]. So, S100P has been noticed as a useful biomarker for pancreatic carcinogenesis [20]. Furthermore, cromolyn has previously been shown to bind specifically to S100P protein, block the interaction with RAGE, and inhibit the tumor growth [13].

In this study, cromolyn was encapsulated in PEGylated liposome with various molar ratio of DSPE-mPEG2000 (Table 1) and the encapsulation efficiency, particle size and zeta potential of PEG-lipo-cro were measured. The best formulation among tested for anti-cancer effect was found to be DPPE: DMPC: DSPC: DSPE-mPEG2000 = 4:1:1:0.1 molar ratio with a mean diameter of 158 nm, zeta potential of −2.23 mV and drug encapsulation efficiency of 28%. When the amount of DSPE-mPEG2000 was increased, the encapsulation efficiency was decreased. It has been known that incorporation of too much amount of PEG-conjugated phospholipid may disrupt the lipid bilayer structure of liposome due to its detergent-like properties [21–23], thereby inhibiting drug encapsulation into the liposome. Under physiological conditions, only smaller liposomes ranging from 100 to 200 nm in diameter could have better opportunities to extravasate through the discontinuous capillaries of tumors and accumulate efficiently in tumor tissues [24,25]. It was also confirmed that the amount of protein adsorption onto the PEGylated liposomes encapsulating cromolyn or gemcitabine was significantly decreased compared to non-PEGylated liposome (Fig. 1) due to the fact that PEG coats the surface of liposomes by making steric barrier to the water, resulting in the repulsive interaction between PEG chain and serum proteins. These findings assured the usefulness of PEGylated liposome as a suitable drug carrier for cancer treatment in vivo.

It was also shown that PEG-lipo-cro exhibited much better anti-proliferative effect on the BxPC-3 cells than on the Panc-1 cells as the BxPC-3 cells contained the endogenous S100P protein but the Panc-1 cells did not (Fig. 2, Fig. S-1). Also, inhibition of NF-κB by PEG-lipo-cro together with gemcitabine was accompanied by marked growth inhibition in BxPC-3 cells (Fig. S-3, Supplementary Pages), as the NF-κB is known to play an important role in oncogenesis and promote the cellular resistance against anticancer therapy in the majority of pancreatic cancer [26,27]. This suggests that cromolyn, an inhibitor of S100P, may have another clinical advantage by making cancer cells more responsive to other anti-tumor agents, such as gemcitabine, through the NF-κB pathway.

In vivo study showed that PEG-lipo-cro exhibited much better tumor growth inhibition than the free cromolyn in BxPC-3-bearing xenograft nude mouse model, presumably due to the increased half-life and the passive targeting effect mediated by the PEGylated liposome (Fig. 3). Moreover, significant tumor growth inhibition without severe adverse effects (including body weight loss, data not presented) was exerted when the combination of PEG-lipo-cro with PEG-lipo-gem was administered. This finding is consistent with the result of TUNEL assay where PEG-lipo-cro enhanced the gemcitabine-induced apoptosis (Fig. 4), though the degree of enhancement is not sufficient enough to fully eradicate the solid tumors as they might express other anti-apoptotic factors as self-protective mechanisms.

In conclusion, this study demonstrates that long circulating PEG-lipo-cro was successfully developed with a promising anti-cancer efficacy for pancreatic cancer in vitro and in vivo, especially when used in combination with PEG-lipo-gem. Further studies need to be done before the clinical application of this formulation for cancer patients.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jconrel.2011.09.066.

References


