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Cytotoxicity of Pure Nanodrugs of SN-38 and Podophyllotoxin Dimers in Human Cancer HepG2, KPL-4, and MCF-7 Cells

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Pure nanodrugs (PNDs) is a newly designed drug materials, which is a carrier-free drug nanoparticles fabricated by the reprecipitation method. In the present study, the cytotoxicity of PNDs consisting of SN-38 dimer and podophyllotoxin dimer with size in the range 30–50 nm was evaluated in human cancer HepG2, KPL-4, and MCF-7 cells. It was observed that the cytotoxicities of PNDs were changed depending on the type of cells. In addition, PNDs coated with polysorbate 80 showed higher activity than free PNDs, which suggests that the surface state of PNDs was the one of the important factors to affect cytotoxicity.

Keywords Pure nanodrugs (PNDs); Reprecipitation method; Cytotoxicity; SN-38; Podophyllotoxin (PPT)

1. Introduction

Nanometer-sized materials have received considerable attention for their potential application as a therapeutic agent for cancer. Nanoparticles including polymer nanocarriers have been widely employed to overcome problems such as drug solubility and target tumors by the enhanced permeability and retention (EPR) effect.[1-3] Recently, we have designed nanometer-sized carrier-free drug nanoparticles (pure nanodrugs: PNDs).[4] In the previous work, two types of PNDs of SN-38 (topoisomerase I inhibitor) dimer and podophyllotoxin (PPT, topoisomerase II inhibitor) dimer have been fabricated using the reprecipitation

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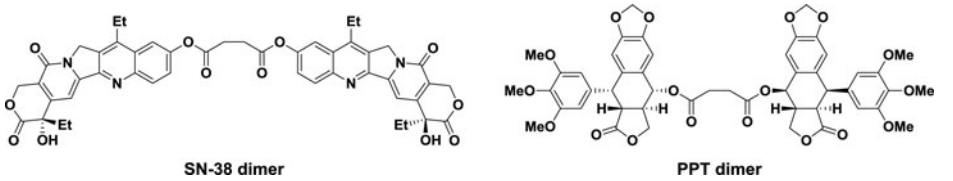

Figure 1. Chemical structures of SN-38 dimer and PPT dimer linked with succinate.

method.[4-5] However, the bioactivity of PNDs only addressed the *in vitro* cytotoxicity in human hepatocellular carcinoma HepG2 cells. In general, the cytotoxicity of drug molecules depends on the source of cancer cells,[6-8] but there are no reports with regard to PNDs. Therefore, in the present work, we aimed to evaluate the cytotoxicity of SN-38 dimer PNDs and PPT dimer PNDs in breast cancer KPL-4[9] and MCF-7[10] cells and compared with that observed in HepG2 cells.

2. Materials and Methods

Fabrication of PNDs: All chemicals were used without further purification. Water was purified up to 18.2 MΩ cm using Arium 611UV (Sartorius Mechtronics Japan K.K.). SN-38 and PPT dimers (Fig. 1) were synthesized by esterification with succinic acid as previously described.[4-5] The nanoparticles of dimers (PNDs) were fabricated by the reprecipitation method.[11] 100 μL of the dimer DMSO solution (5 mM) was rapidly injected into vigorously stirred pure water (10 mL) by a microsyringe. Polysorbate 80-coated PNDs were fabricate using DMSO containing polysorbate 80 (1% w/v).

Characterization of PNDs: The resulting nanoparticles were evaluated using a dynamic light scattering instrument (DLS: Zetasizer Nano series Nano-ZS; Malvern Instruments, Ltd.) and scanning electron microscopy (SEM: JSM-6700F; JEOL).

Cell viability assay: HepG2 and MCF-7 cells were purchased from RIKEN Cell Bank and the Cell Resource Center for Biomedical Research, Tohoku University, respectively. KPL-4 cells was kindly provided by Dr. J. Kurebayashi (Kawasaki Medical School, Japan). All cells were cultured under 5% CO_2 at 37 °C. HepG2 cells was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 30 U/mL of penicillin, and 30 μg/mL of streptomycin. KPL-4 and MCF-7 cells were cultured in DMEM containing 5% and 10% FBS, respectively. HepG2, KPL-4, and MCF-7 cells were seeded in 96-well plates (2×10^4 cells/well) and incubated 24 h. Then, the culture medium was removed and 100 μL of medium containing PNDs or water-soluble derivatives were added. After 48 h, the number of viable cells were evaluated using the Cell Counting Kit-8 (DOJINDO) and a Microplate Reader (iMark; Bio-Rad Laboratories, Inc.). Cell viabilities were normalized to ($OD_{450} - OD_{620}$) for the untreated cells. Assays were performed in triplicate.

3. Results and Discussion

PNDs were prepared by the reprecipitation method. The resulting nanoparticles were observed by SEM. The average particle sizes of SN-38 dimer nanoparticles (SN-38-PNDs) and PPT dimer nanoparticles (PPT-PNDs) were 31 ± 7 nm and 52 ± 14 nm, respectively (Fig. 2).

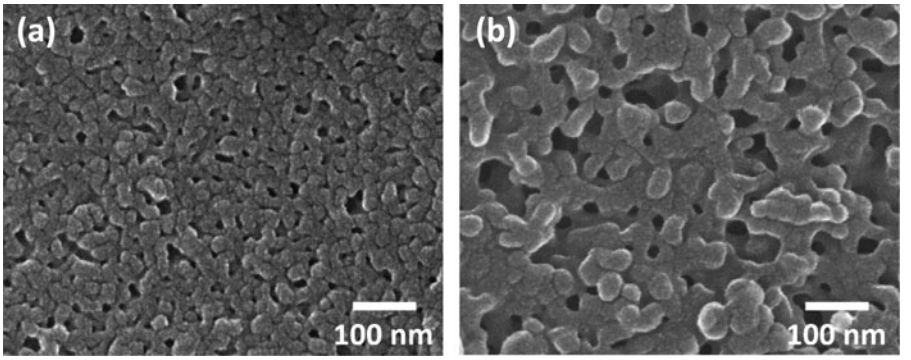

Figure 2. SEM images of SN-38-PNDs (a) and PPT-PNDs (b).

HepG2, KPL-4, and MCF-7 cells were treated with the aqueous dispersions of SN-38-PNDs and PPT-PNDs (0.1–10 μM) for 48 h, and the number of viable cells was determined using a WST-8 assay. Previously, the half-maximal inhibitory concentration (IC_{50}) of SN-38-PNDs and PPT-PNDs were observed to be approximately 1 μM in HepG2 cells.[5] Therefore, we chose this concentration to evaluate the activity among different cancer cell lines. As shown in Fig. 3, the cytotoxicity of SN-38-PNDs and PPT-PNDs in HepG2 and KPL-4 cells were much greater than that of irinotecan and etoposide, which are a water-soluble prodrug and analog of SN-38 and PPT, respectively. By contrast, the cytotoxicity was low in MCF-7 cells. Interestingly, the SN-38-PNDs showed higher cytotoxicity than PPT-PNDs in HepG2 cells, while SN-38-PNDs showed lower cytotoxicity than PPT-PNDs in KPL-4 cells. We hypothesized that this cytotoxic behavior of HepG2 and KPL-4 cells was caused by the different amounts of PND uptake of into the cells related to the surface state of the PNDs.

In order to evaluate the effect of surface state of PNDs on cellular uptake, we coated SN-38-PNDs and PPT-PNDs with polysorbate 80. Fig. 4 shows the comparison of the cytotoxicity between free and polysorbate 80 (0.01% w/v)-coated PNDs in HepG2 cells. Polysorbate 80-coated PNDs had higher cytotoxicity than free PNDs. This is likely because polysorbate 80 had adsorbed on the surface of PNDs. Hence, this result suggested that the surface state of the PNDs affected their uptake efficiency into cells.

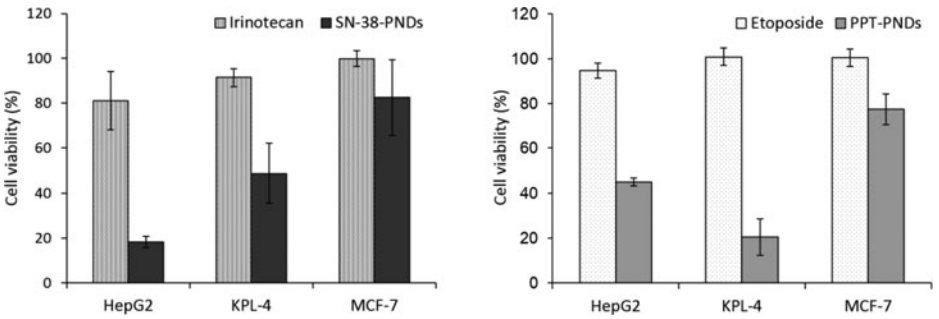

Figure 3. *In vitro* cytotoxicity of SN-38-PNDs and Irinotecan (a), PPT-PNDs and Etoposide (b) in HepG2, KPL-4, and MCF-7 cell. Each concentration is 1 μM. All results are indicated by the mean $\pm$ standard deviation ($n = 3$).

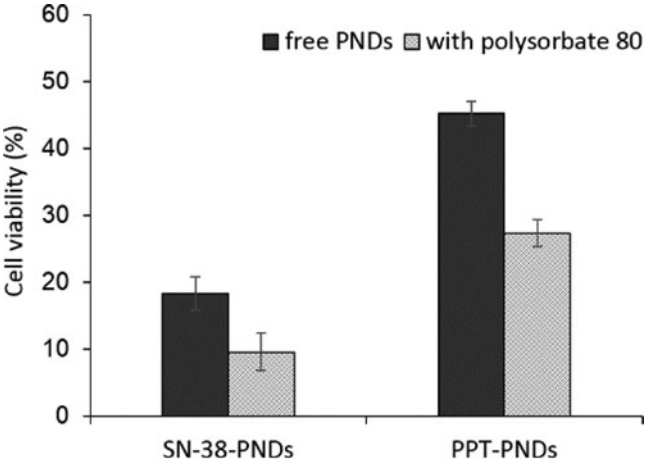

Figure 4. *In vitro* cytotoxicity of free PNDs and polysorbate 80-coated PNDs in HepG2 cells. Each concentration is 1 μM. All results are indicated by the mean $\pm$ standard deviation ($n = 3$).

4. Conclusions

We investigated the cytotoxicity of SN-38-PNDs and PPT-PNDs in HepG2, KPL-4, and MCF-7 cells. The relative cytotoxicities were SN-38-PNDs > PPT-PNDs in HepG2 cells but SN-38-PNDs < PPT PNDs in KPL-4 cells. In addition, it was revealed that the surface state of PNDs was one of the important factors to affect cytotoxicity.

Acknowledgments

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