PEGylation of Liposome Decreases the Susceptibility of Liposomal Drug in Cancer Photodynamic Therapy

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For the purpose of the avoidance of reticuloendothelial system (RES)-trapping, liposome entrapped benzoporphyrin derivative monoacid ring A (BPD-MA), which is used for cancer photodynamic therapy (PDT), was modified with polyethylene glycol (PEG-LipBPD-MA). Tumor accumulation of BPD-MA at 3 h after injection with PEG-LipBPD-MA in Meth A-sarcoma-bearing mice was significantly higher than that after injection with non-modified liposomal BPD-MA (Cont-LipBPD-MA) as expected. On the contrary, significant tumor growth suppression after PDT was observed only for Cont-LipBPD-MA but not for PEG-LipBPD-MA. Thus, PEGylation enhances the passive targeting of liposomal BPD-MA in tumor, but decreases the susceptibility of the drug in PDT.

Key words: polyethylene glycol (PEG); liposome; photodynamic therapy (PDT); targeting; drug delivery system (DDS)

Photodynamic therapy (PDT) is a modality of cancer treatment that uses a combination of photosensitizer and tissue-penetrating laser light. After laser irradiation, singlet oxygen is produced and induces cytotoxicity. Benzoporphyrin derivative monoacid ring A (BPD-MA) is a second-generation photosensitizer, and is clinically used for the treatment of age-related macular degeneration. Since BPD-MA is a hydrophobic property, it is formulated as a liposomal drug. In the previous study, we established rather stable liposomal BPD-MA, where liposome was composed of dipalmitoylphosphatidylcholine (DPPC), palmitoyloleoylphosphatidylcholine (POPC), dipalmitylophosphatidylglycerol (DPPG), cholesterol, and BPD-MA (10/10/2.5/10/0.3 as molar ratio).¹

In the present study, to enhance the accumulation of liposomal BPD-MA in tumor tissues, BPD-MA liposome was modified with polyethylene glycol (PEG), since PEG-liposomes are known to have long-circulating characteristics after systemic injection, and to accumulate passively in tumor in tumor-bearing animals.²,³ As a result, we observed the enhanced accumulation of BPD-MA in tumor tissue by PEGylated liposome, but the therapeutic efficacy was unexpectedly rather decreased by PEG-modification.

BPD-MA-entrapped PEG-liposome (PEG-LipBPD-MA) and BPD-MA-entrapped non-modified liposome (Cont-LipBPD-MA) were composed of DPPC, POPC, cholesterol, DPPG, and BPD-MA (10/10/2.5/0.3 as molar ratio), and were sized at 100-nm in diameter by extrusion technique. In animal experiments, the animals were cared for according to the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka.

For the purpose of avoiding reticuloendothelial system (RES)-trapping of macromolecular drugs, polymer drugs, and drug carriers in systemic usage, PEGylation has been widely applied, including polymer conjugated photosensitizers for PDT.⁴,⁵) The feature of long-circulation causes enhanced accumulation of such drugs and carriers in tumor tissues because angiogenic vasculature in tumor tissue is quite leaky and macromolecules are easily accumulate in the interstitial tissues of the tumor due to the enhanced permeability and retention (EPR) effect.⁶,⁷)

Therefore, we first examined BPD-MA accumulation in tumor at 3 h after injection of Cont-LipBPD-MA and PEG-LipBPD-MA in tumor-bearing mice. Meth A-sarcoma cells (1×10⁶cells/0.2 ml) were injected s.c. into BALB/c mice (Japan SLC). Seven days after implantation of the tumor, the tumor-bearing mice were injected i.v. with liposomal BPD-MA (2 mg/kg as BPD-MA). The mice were sacrificed at 3 h after injection of the liposomal BPD-MA. Then, BPD-MA was extracted from the tumor, and analyzed by using HPLC (Shimadzu, Japan). PEGylation of liposomes actually greatly enhanced tumor accumulation of BPD-MA as expected (Fig. 1).

Next, we examined the suppression of tumor growth after PDT by use of these liposomal formulations. Liposomal BPD-MA (0.5 mg/kg as BPD-MA, instead of 2 mg/kg) was injected into tumor-bearing mice similarly prepared to the distribution assay at day 7 after tumor implantation. PDT treatment was performed by irradiation at the tumor site with 689 nm laser light (150 J/cm², 0.25 W) at 3 h after injection of liposomal BPD-MA. At day 16 after tumor implantation, tumor sizes were examined (Fig. 2).

The result is unexpected and quite interesting, that tumor growth suppression was more obvious for Cont-LipBPD-MA-injected group after treatment of PDT than for PEG-LipBPD-MA. The suppression of tumor growth was more obvious for Cont-LipBPD-MA-injected group after treatment of PDT than for PEG-LipBPD-MA.
LipBPD-MA-injected group, although the total amount of BPD-MA in tumor tissue is more than 5-fold higher for the latter than former as shown in Fig. 1. Corresponding to the tumor growth suppression, the mean lifetime of tumor-bearing mice \( n = 5 \) was 30.6, 37.0, and 29.4 d for non-treatment, Cont-LipBPD-MA-PDT, and PEG-LipBPD-MA-PDT groups, respectively.

Recently, it was revealed that anti-cancer drugs administered with repeated low dose, namely metronomic dosing, damage angiogenic endothelial cells as well as tumor cells.8,9) Interestingly, PEG-liposomal adriamycin, Doxil, is thought to damage angiogenic cells besides its direct toxicity against tumor cells, because tumor tissues including neovessels are exposed to lower dose of ADM for longer time than the case of free ADM-treatment: Long-circulating liposomal anti-cancer drugs may behave similar to those administered metronomic dosing. This evidence strongly suggests that liposomal carriers release BPD-MA slowly at the tumor site. Furthermore, PEG in PEG liposome may protect direct interaction of liposome with tumor cells. On the contrary, non-coated liposome may contact more easily with tumor cells and deliver BPD-MA to the cells. Alternatively, PEG protects the interaction of the liposome with macrophages in the interstitial space of tumor cells, that may release BPD-MA.

Since the lifetime of active oxygen is quite short, BPD-MA taken up by tumor cells may damage the cells, but that in interstitial tissues in tumor as liposomal form may not. PEG-liposomes encapsulating anti-cancer drugs are useful for passive targeting, and the drugs accumulated in tumor tissue act for longer period time to tumor cells. In PDT, however, only the photosensitizers taken up by tumor cells damage tumor cells when laser irradiation is performed. In the present study, we scheduled 3 h for allowing liposomal BPD-MA to interact tumor cells. Therefore, PEGylation effect was not positively observed but reduced the susceptibility. It is possible, if we allow longer period of time for the interaction, PEGylation effect becomes positive.

We previously observed that glucuronate-modified liposomal BPD-MA strongly suppressed tumor growth in tumor-bearing mice after laser irradiation at 5 h post-injection.10) In this case, the enhanced therapeutic efficacy may be explained by that 5 h is enough to allow BPD-MA-delivering to tumor cells, and/or that glucuronate-modification does not protect the interaction of liposome to tumor cells.

In conclusion, although PEGylation of liposome enhances passive accumulation of liposomal drugs in tumor tissues, it does not always beneficial for therapy, especially in case of PDT, in which fast accumulation of photosensitizers in tumor cells is critical. However, it is possible that the conjugation of some ligands or antibodies specific for target cells to PEG-liposome overcome the problem.

REFERENCES