Radiolabeled Europium Loaded Theranostic Liposomal Nanoparticles for Effective Radioisotope induced Photodynamic Therapy

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Purpose: Photodynamic therapy (PDT) is conducted by a photosensitizer (PS) and a light which can excite the PS to remove the target tumor tissues locally through reactive oxygen species (ROS) production. Radioisotope induced luminescence such as Cerenkov luminescence could be used for PDT, which has an advantage of independence of external light. However, Cerenkov luminescence is a relatively weak light which leaves uncertainty for clinical translation of this approach. Meanwhile, the interaction between radioisotope and nanoparticles with high Z materials such as Europium (Eu) can emit radioluminescence, which also can be used for PDT. Herein, we developed radiolabeled Eu loaded liposomal nanoparticles for enhanced PDT and *in vivo* imaging.

Methods: The Eu^{3+} ion was chelated to diethylenetriaminepentaacetic acid (DTPA) (Eu-DTPA). Victoria blue-BO (VBBO) was chosen for PS. Liposome was synthesized by a self-assembly method using phosphatidylcholine (PC) derivatives. Eu-DTPA and VBBO were loaded into the hydrophilic core part of the structure during the self-assembly method to make Eu loaded (Eu-Lipo), Eu and VBBO loaded (Eu/VBBO-Lipo), and VBBO loaded liposome (VBBO-Lipo). The liposome nanoparticles were further modified by using NOTA and PEG for radiolabeling and long circulation in vivo. Eu-Lipo, VBBO-Lipo, and Eu/VBBO-Lipo were radiolabeled with ^{64}Cu (^{64}Cu-Eu-Lipo, ^{64}Cu-VBBO-Lipo, ^{64}Cu-Eu/VBBO-Lipo) and tested for luminescence using IVIS. *In vitro* ^{64}Cu induced PDT was conducted. ^{64}Cu-Eu/VBBO-Lipo was injected intravenously to mouse tumor models, and the positron emission tomography (PET) images were acquired at 0, 4, 24, and 48 hours after the injection.

Results: The hydrodynamic size of self-assembled Eu/VBBO-Lipo was 80±15 nm. Eu/VBBO-Lipo had high stability for seven days in the physiological conditions. ^{64}Cu-Eu-Lipo (Radioluminescence) showed two-fold higher luminescence intensity than ^{64}Cu (Cerenkov luminescence) ($P < 0.0001$). ^{64}Cu-VBBO-Lipo showed a lower luminescence intensity than ^{64}Cu because of Cerenkov luminescence energy transfer (CLET), while ^{64}Cu-Eu/VBBO-Lipo showed a lower luminescence intensity than ^{64}Cu-Eu-Lipo because of radioluminescence energy transfer (RET). RET efficiency (~27.6% of RL) was about 6 times higher than CLET efficiency (~4.7% of CL) ($P < 0.0001$). *In vivo* PET imaging demonstrated that ^{64}Cu-Eu/VBBO-Lipo showed a substantially long blood pool circulation with circulation half-life of 10.4 hours and a sufficient tumor uptake at 24 hours after the injection (~ 20%ID/g). ROS generation and PDT effect were significantly higher in ^{64}Cu-Eu/VBBO-Lipo than ^{64}Cu-VBBO-Lipo ($P < 0.001$, and $P < 0.001$).

Conclusions: ^{64}Cu-Eu/VBBO-Lipo demonstrated a bright radioluminescence, effective RET, ROS production, and PDT effects, which were superior to Cerenkov induced PDT using ^{64}Cu-VBBO-Lipo. Furthermore, ^{64}Cu-Eu/VBBO-Lipo showed long circulation and effective passive targeting in mouse tumor model. Based on these findings, ^{64}Cu-Eu/VBBO-Lipo could be successfully utilized for radioisotope induced PDT.