Open-shell Nanosensitizers for Glutathione-Responsive Cancer Sonodynamic Therapy

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Introduction Sonodynamic therapy (SDT) is the stimulation of sonosensitizing molecules with ultrasound for cell killing. Herein we developed/evaluated a nanosensitizer (**NS**) that can spare normal tissue, utilizing the sonosensitive properties of Cu(I) and Cu(II), as well as the ability of glutathione (GSH, present in many tumors) to reduce Cu(II) to Cu(I), where Cu(I) is highly sonosensitive but Cu(II) is not. We constructed Cu(II) nanosensitizers (*i.e.* Cu(II)NS) using porphyrins, Cu(II), and polyethylene glycol (PEG), where PEG can increase the circulation half-life and tumor accumulation of these NS.

Methods Cu(II)NS was synthesized from tetrakis carboxyphenyl porphyrin (TCPP)-PEG nanoparticles, which can chelate Cu(II). The ratio of Cu(II)/Cu(I) in the NS before and after exposure to GSH was measured. Biodistribution of Cu(II)NS was studied with PET after intrinsic ^{64}Cu-labeling,[1,2] and 4T1 tumor-bearing mice were injected with ^{64}Cu(II)NS and scanned at multiple time points post injection (p.i.). Ex vivo PET of organs was performed at 72 h p.i. Cu(II)NS with ultrasound treatment was assessed in vitro on 4T1 cells and measurements focused on intracellular reactive oxygen species (ROS) and cell apoptosis. Cell uptake and reduction of Cu(II)NS was measured by fluorescence, as Cu(II)NS does not exhibit fluorescence but Cu(I)NS does. In vivo treatment was evaluated in 4T1 tumor-bearing mice (n=5/group).

Results Cu(II)/Cu(I) ratio in Cu(II)NS and Cu(I)NS was found to be 0.675 and 0.254, respectively, indicating that GSH can reduce Cu(II) to Cu(I) in Cu(II)NS. PET scans revealed that Cu(II)NS had blood half-life of ~10 hours, allowing ample time for GSH response. High and uniform tumor distribution of NS was observed, corroborated by ex vivo imaging. In vitro stimulation of Cu(II)NS with ultrasound showed that Cu(II)NS can generate much more singlet molecular oxygen (a ROS) than ultrasound alone, resulting in much higher cell apoptosis. In vitro fluorescence study showed that there was high cellular uptake of Cu(II)NS and subsequently high presence of Cu(I)NS due to reduction by intracellular GSH. In vivo studies also demonstrated increased fluorescent intensity in 4T1 tumor over time, indicating presence of Cu(I)NS, while in normal tissue there was little fluorescence or presence of Cu(I)NS. Among all groups, Cu(II)NS with ultrasound most effectively inhibited the growth of 4T1 tumors.

Conclusion We synthesized Cu(II)NS, a GSH-responsive NS which can avoid harmful side effects to normal tissue while inducing apoptosis to tumor cells, with a long blood $t_{1/2}$ and favorable whole body and tumor biodistribution.[3] Treatment with Cu(II)NS and ultrasound led to effective inhibition of 4T1 tumor growth. PET and florescence imaging enabled facile and accurate measurement of the biodistribution and response of the NS to tumor microenvironment.

References

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