Monitoring Nanoemulsion-Based Oxygen Delivery using Non-Invasive Tumor Hypoxia Imaging

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ABSTRACT SUMMARY

Oxygen-containing perfluorooctyl bromide (PFOB) nanoemulsions were prepared for delivery of oxygen to hypoxic tumors. PFOB with high oxygen solubility comprised the core of the nanoemulsion and was stabilized by phospholipids and polysorbate 60. Co-administration of oxygenated PFOB nanoemulsion and 2-nitroimidazole-ICG to tumor-bearing mice resulted in a significant signal reduction of the hypoxia probe 2-nitroimidazole-ICG in tumors, indicating the successful oxygen delivery to tumors.

INTRODUCTION

Most solid tumors contain a tumor-specific microenvironment that is characterized by low oxygen partial pressure (pO₂). It has been known that tumor hypoxia alters the pattern of gene expression leading to more aggressive behavior with increased metastatic potential and resistance to chemotherapy and radiotherapy. The goal of the project is to image tumor hypoxia and pre-condition the hypoxic tumors using O₂-carrying nanoemulsions in pre-clinical human lung tumor models to improve the effectiveness of chemo- and radiation therapy.

Perfluorocarbons, perfluorooctyl bromide (PFOB) in particular, have been developed as oxygen carriers due to their high capacity for dissolving oxygen and good safety profile. A nanoemulsion system was designed to deliver O₂-carrying PFOB to tumors through the enhanced permeation and retention (EPR) effect. Nitroimidazoles are widely used as molecular probes for tumor hypoxia due to their specific binding to proteins expressed in a low pO₂ environment. Novel dye-conjugates that link a 2-nitroimidazole moiety to an ICG derivative were synthesized as hypoxia probes. The subsequent reduction of tumor hypoxia was monitored by near infrared (NIR) fluorescence imaging using our novel 2-nitroimidazole-ICG fluorescent hypoxia probe.

EXPERIMENTAL METHODS

20% v/v PFOB nanoemulsion was prepared with phosphotidylcholine, cholesterol and Tween 60. The nanoparticles were characterized by TEM and Nanosight Particle Characterization System for size distribution and zeta potential.

Optical imaging was carried out using Severe combined immunodeficiency (SCID) mice with human lung cancer A549 cell line implanted subcutaneously. The hypoxia probe 2-nitroimidazole-ICG dissolved in 9% w/v sucrose aqueous solution (25 μM) was injected intravenously to 20 mice (100 μL/mouse). The tumor fluorescent signal was monitored within 48 hours using a IVIS imaging system. The mice were separated into 5 groups (4 in each group). Six days after the hypoxia probe injection, a second i.v. injection was followed to each group including (1) 2-nitroimidazole-ICG hypoxia probe; (2) 2-nitroimidazole-ICG hypoxia probe mixed with PFOB nanoemulsion (oxygenated); (3) 2-nitroimidazole-ICG hypoxia probe mixed with PFOB nanoemulsion (non-oxygenated); (4) PFOB nanoemulsion (oxygenated) followed 25 min later with 2-nitroimidazole-ICG hypoxia probe; (5) PFOB nanoemulsion (non-oxygenated) followed 25 min later with 2-nitroimidazole-ICG hypoxia probe.
RESULTS AND DISCUSSION

The mean particle size and zeta potential of PFOB nanoemulsion were characterized as 198 nm and -16.2 mV, respectively.

![Image](image.png)

Figure 1. Number based size distribution(A) and TEM image (B) of PFOB nanoemulsion.

Co-administration of oxygenated PFOB nanoemulsion and 2-nitroimidazole-ICG significantly reduced the accumulation of hypoxia probe on tumor, indicating the successful delivery of oxygen to tumor (Figure 2A, B and C). The delivered oxygen has a short term effect (Figure 2 D and E)

![Image](image.png)

Figure 2. Tumor fluorescence (radiation efficiency) as a function of time flowing initial administration of the 2-nitroimidazole-ICG probe(red line) and various treatments with PFOB nanoemulsions (blue line) 6 days later.

CONCLUSION

The PFOB nanoemulsion was able to deliver oxygen to tumors. The efficacy of chemotherapy and radiation therapy is expected to be improved by being co-administered with the oxygenated PFOB nanoemulsion.

REFERENCES


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