Liposomal delivery of radionuclides for cancer diagnostics and radiotherapy: From material development to in vivo applications using positron emission tomography (PET) imaging

Molecular imaging is increasingly being used as an integrated discipline in designing radiotherapeutic agents and diagnostic imaging agents, and in developing drugs in general. In recent years, the use of the radionuclide and positron emitter copper-64 (64Cu) has become increasingly important, as the use of positron emission tomography (PET) scanners for molecular and diagnostic imaging has become more attractive. Furthermore, the importance of molecular and diagnostic imaging in nanotechnology has also been recognized, and significant research has been conducted on radiolabeled liposomes for scintigraphy and single photon emission computed tomography (SPECT) imaging. Preclinical as well as clinical SPECT studies on radiolabeled liposomes have contributed with valuable information on the pharmacokinetics of liposomes during several liposomal drug developments. SPECT has lower detection sensitivity compared to PET, and developing new radio-labeling and loading methods, and designing liposomes useful in PET imaging are therefore of great importance.

The first two sections of this thesis (Introduction and Project I) review current liposomal radio-labeling and loading strategies, and describe the use of 64Cu in PET imaging. Article I and Patent I present our work focused on developing a remote (active) loading technique using a new lipophilic chelator (2-hydroxyquinoline) to load 64Cu into preformed (pre-manufactured) liposomes. We optimized the remote loading technique through several liposomal loading experiments and isothermal titration calorimetry (ITC) measurements. Various chelators, ionophores and lipophilic chelators were tested at different pH and temperature conditions.

Liposomes passively accumulate in tumors due to the enhanced permeability and retention (EPR) effect. In Article I, an in vivo study is presented, where passive tumor accumulation of 64Cu loaded liposomes (64Cu-liposomes) in tumor-bearing mice was quantified directly by PET and computed tomography (CT) imaging. Furthermore, Article I present an evaluation and quantitative measurement of the biodistribution of 64Cu-liposomes in healthy and tumor-bearing mice.

Project II summarizes considerations required in designing diagnostic liposomal radiotracers such as liposomal properties (e.g. particle size and poly(ethylene glycol) PEG coating), importance of background clearance in diagnostic imaging, and radiation exposure during PET scanning and internal radiotherapeutic applications. The circulation half-life of liposomes can be modulated by tuning the particle size and surface coating by varying the degree of PEG. Article II presents our work comparing two liposomal formulations coated with PEG (5 mol% PEG and 10 mol% PEG) in vivo in tumor-bearing mice. In addition, we perform remote loading experiments of the radionuclide, lutetium-177, (177Lu) into preformed liposomes useful in internal radiotherapy. Furthermore, Article II presents a dosimetric evaluation of PEGylated 64CuII liposomes as clinical PET radiotracers and PEGylated 177Lu-liposomes useful in internal tumor radiotherapy.

While liposomes passively accumulate in tumor sites due to the EPR-effect, active targeting strategies using ligands directed towards over-expressed receptors on tumor cells can enhance tumor accumulation of liposomes. Project III briefly describes some active tumor-targeting strategies that have been tested with liposomes. In addition, Project III describes over-expressed somatostatin receptors (SSTRs) in neuroendocrine (NE) tumors and how SSTRs can be targeted with radiolabeled somatostatin analogs in imaging and radiotherapy of NE tumors in cancer patients. Article III presents our work, in which we investigate the capability of 64Cu-liposomes to actively target NE tumors when a somatostatin analog, octreotate (TATE), as targeting ligand is covalently attached to the distal end of DSPE-PEG2000. We also compare the biodistribution of PEGylated 64Cu-liposomes with and without TATE, and their ability to image NE tumors in tumor-bearing mice using PET. Further, we compare the liposome tumor accumulation and imaging capability with that of the radiolabeled somatostatin analog 64Cu-DOTA-TATE.

During the past 30 years, ionophores or lipophilic chelators have commonly been used for remotely loading radionuclides into liposomes. During the optimization of our remote loading method (Project I), we discovered a much simpler and even more efficient approach to load the radionuclides 64Cu, indium-111 (111In) and 177Lu into preformed liposomes, a so-called “unassisted” loading, excluding any use of ionophores and lipophilic chelators. Project IV presents results from this invention (Patent II), where a presentation of various parameters affecting the efficiency of the unassisted loading method is given.

Section 5 summarizes the regulatory requirements governing quality assurance and considerations during development of a liposomal PET radiopharmaceutical for clinical use. In addition, the opportunity to use larger animals as clinical cancer patients for improving 64Culiposomes as PET imaging agents is briefly described followed by a short summary of possible clinical applications of a liposomal PET radiotracer.

Section 6 summarizes the work of this thesis and brings the work into perspective.