SPECT Imaging of Cell Death Using $^{99m}$Tc-labeled C2A Domain of Synaptotagmin I

The C2A domain of Synaptotagmin I binds both apoptotic and necrotic cells by interacting with a common molecular marker, phosphatidylserine (ptds). These forms of cell death constitute the primary consequence of acute myocardial infarction and anti-tumor chemotherapy. Fluorescently and radiolabeled C2A as targeted molecular probes have been shown to enable non-invasive imaging of cell death, using optical imaging and single-photon planar imaging, respectively. In collaborating with Dr. Ming Zhao at the Medical College of Wisconsin, we are studying the characterization of the in vivo dynamic-imaging properties of $^{99m}$Tc-C2A in detecting cell death using advanced dynamic SPECT imaging techniques. First, we have preliminarily characterized the pharmacokinetics and distribution of $^{99m}$Tc-C2A in rats with reperfused acute myocardial infarction. Subsequently, we are studying the possibility of quantification or semi-quantification of $^{99m}$Tc-C2A uptake at the acute infarct site and to correlate the level of focal uptake with the extent of myocardial cell death. Second, we are investigating the in vivo targeting properties of $^{99m}$Tc-C2A in evaluating anti-tumor effects of Taxol in xenografted SCID mice with breast cancer.

C2A-GST was labeled with $^{99m}$Tc via 2-iminothiolane thiolation. Myocardial ischemia was induced by ligating the left anterior descending coronary artery for 30 minutes, followed by 120 minutes of reperfusion in six Sprague-Dawley rats. $^{99m}$Tc-C2A (185 MBq) was intravenously injected via a jugular vein catheter. Immediately after injection, dynamic cardiac images in list-mode acquisition were recorded over a 2-hour period using FastSPECT II to collect in vivo radiotracer kinetics. Tomographic images showed a focal radioactive accumulation (hot spot) in the lateral and anterior wall of the left ventricle. The hot spot was initially visualized 10 minutes after injection and persisted on the 2-hour images in all three hearts. The hot-spot radioactivity uptake reached a plateau within 1 hour after radiotracer injection and experienced no washout up to the end of the 2-hour study. At 2 hours after injection, the average ratio of the hot spot to remote viable myocardium was 4.52 ± 0.24, and the infarct-to-lung ratio was 8.22 ± 0.63. The uptake of $^{99m}$Tc-C2A-GST in the ischemic area-at-risk was confirmed by post-mortem triphenyltetrazolium chloride (TTC) staining and digital autoradiography analysis. The results suggest that $^{99m}$Tc-C2A may be clinically useful in detecting and quantifying acute irreversible myocardial cell loss, including apoptosis and oncrosis.

In an imaging study of $^{99m}$Tc-C2A tumor targeting, xenografted breast tumors were grown by implanting $5 \times 10^6$ human MCF7 breast-cancer cells into the thigh or flank area of SCID mice. Mice were monitored in a sterile environment for palpable tumors. Six tumor-carrying mice with single-dose Taxol (21 mg/kg) therapy have been studied. $^{99m}$Tc-C2A images were collected using FastSPECT before and after Taxol treatment. The preliminary results showed that the chemotherapeutic response induced by Taxol is detectable by in vivo $^{99m}$Tc-C2A imaging.

To date, an abstract regarding in vivo dynamic imaging of myocardial cell death using $^{99m}$Tc-C2A has been submitted to the 54th Annual Meeting of the Society of Nuclear Medicine [1]. A peer-reviewed manuscript is in preparation [2].
