

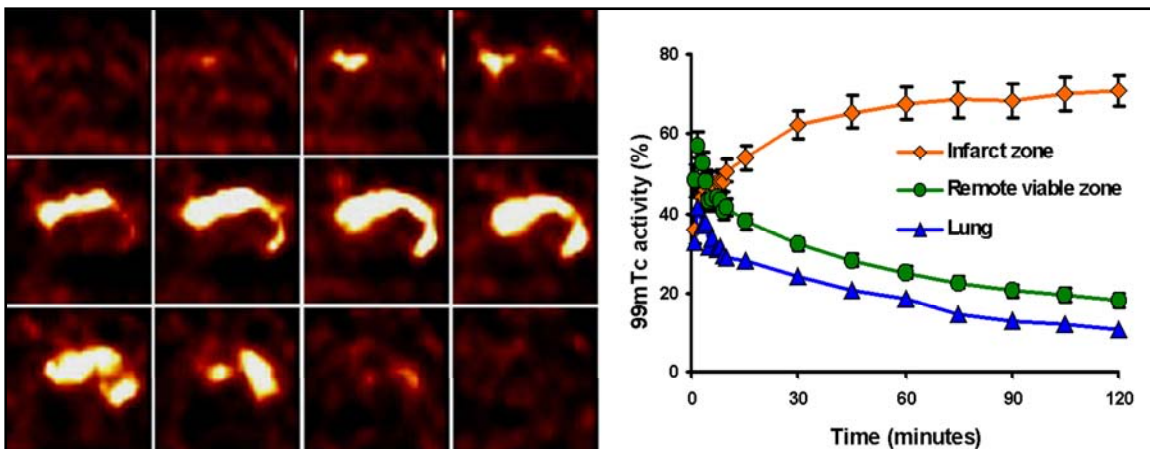
## SPECT Imaging of Cell Death Using $^{99m}\text{Tc}$ -labeled C2A Domain of Synaptotagmin I

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  $^{99m}\text{Tc}$ -C2A in detecting cell death using advanced dynamic SPECT imaging techniques. First, we have preliminarily characterized the pharmacokinetics and distribution of  $^{99m}\text{Tc}$ -C2A in rats with reperfused acute myocardial infarction. Subsequently, we are studying the possibility of quantification or semi-quantification of  $^{99m}\text{Tc}$ -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  $^{99m}\text{Tc}$ -C2A in evaluating anti-tumor effects of Taxol in xenografted SCID mice with breast cancer.

C2A-GST was labeled with  $^{99m}\text{Tc}$  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.  $^{99m}\text{Tc}$ -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  $4.52 \pm 0.24$ , and the infarct-to-lung ratio was  $8.22 \pm 0.63$ . The uptake of  $^{99m}\text{Tc}$ -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  $^{99m}\text{Tc}$ -C2A may be clinically useful in detecting and quantifying acute irreversible myocardial cell loss, including apoptosis and oncosis.

In an imaging study of  $^{99m}\text{Tc}$ -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.  $^{99m}\text{Tc}$ -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*  $^{99m}\text{Tc}$ -C2A imaging.

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



**Figure 1. Left:** Representative tomographic  $^{99m}\text{Tc}$ -C2A images (sagittal slices) collected by FastSPECT II 2 hours post-injection in a rat heart with 30-minute ischemia and 240-minute reperfusion.

**Right:** Normalized time-activity curves of  $^{99m}\text{Tc}$ -C2A in infarct zone and remote viable zone in the rat hearts with ischemia-reperfusion and lungs.

1. M. Zhao, Z. Liu, X. Zhu, L. R. Furenlid, Y-C. Chen, and H. H. Barrett, *In vivo* dynamic imaging of myocardial cell death using  $^{99m}\text{Tc}$ -labeled C2A domain of Synaptotagmin I in a rat model of ischemia and reperfusion, Submitted to the 54<sup>th</sup> Annual Meeting of the Society of Nuclear Medicine, Washington DC, 2007.

2. Z. Liu, M. Zhao, X. Zhu, L. R. Furenlid, Y-C. Chen, and H. H. Barrett, Detection of myocardial cell death using  $^{99m}\text{Tc}$ -labeled C2A domain of Synaptotagmin I in ischemic-reperfused rat hearts, *Nucl. Medi. Biol.*, to be submitted, 2007.