PhD Thesis title: 'Molecular imaging of spatio-temporal distribution of angiogenesis in a hindlimb ischemia model and diabetic milieu.**'**

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ABSTRACT: In the current thesis, spatio-temporal evaluation of the endogenous angiogenic response to ischemia was performed. After vascular occlusion, ischemic angiogenesis is an important reparative mechanism and can ameliorate the outcome of ischemic disease. Diabetic foot ulcers affect almost 15% of diabetic patients and are the leading cause of amputations worldwide. Diminished blood flow because of atherosclerotic occlusive disease of the peripheral arteries of diabetic patients, in conjunction with anatomic and functional microcirculatory impairments contribute to development of trophic ulcerations, infections and gangrene of the lower extremities, frequently requiring amputation of the leg. Numerous studies have confirmed the impaired post-ischemic angiogenesis in diabetes. Consequently, wound healing patterns are disturbed in diabetes mainly due to decreased ischemia-driven angiogenesis. Integrin $\alpha_v\beta_3$ is a promising imaging target of angiogenic activity which is up-regulated on activated endothelial cells but not on quiescent ones. Molecular imaging (MI) of $\alpha_v\beta_3$ integrin expression with the aid of a dedicated high resolution gamma camera, is a very sensitive imaging approach for the evaluation of angiogenesis in the rabbit hindlimb ischemia model. Furthermore, diabetes mellitus (DM) was induced, to study the effects of this pathology on the spatio-temporal distribution of angiogenesis.

During the first part of the study the DM experimental protocol was investigated in order to find the appropriate protocol for the induction of long-term diabetic animal model, as it is a methodology that has not yet been standardized. At the same time a cohort of animals underwent endovascular embolization for the establishment of hindlimb ischemia and were imaged with the aid of a MI radiotracer technique in

order to deal with unresolved issues and establish the imaging protocol. The study included seven New Zealand White rabbits that underwent unilateral percutaneous endovascular embolization of the femoral artery, for the establishment of hindlimb ischemia that triggers the endogenous process of collateralization. The contralateral limb was not embolized and served as a control. The employed radiotracer for angiogenesis imaging, was a ^{99m}Tc labeled cyclic RGD peptide [c RGDfk-His]-^{99m}Tc that binds specifically to $\alpha_v\beta_3$ integrin via the three amino acid sequence Arginine-Glycine-Aspartic acid or RGD. Image acquisition was performed with a high resolution gamma camera and all animals underwent molecular imaging on the 3rd and the 9th day post-embolization. In all animals Digital Subtraction Angiography (DSA) was performed on the 9th day post-embolization.

Angiogenesis was successfully detected using a ^{99m}Tc labeled cyclic RGD peptide molecular imaging technique and was significantly more pronounced in the ischemic compared to normal limbs, both at day 3 and day 9 after embolization. The peak of the phenomenon was detected at day 9. Increased mean vessel length in the normal compared to the ischemic limb demonstrates that although angiogenesis is pronounced at day 9, arteriogenesis is not sufficiently pronounced and that the phenomenon of arteriogenesis has just initiated.

References to author publications that relate specifically to the dissertation:

K. Tsioupinaki, S. Spiliopoulos, D. Karnabatidis, G. Loudos, G.C. Nikiforidis, G.C. Kagadis, “Molecular imaging for the in vivo monitoring of angiogenesis in a hindlimb ischemia animal model,” accepted for publication in Journal of Frontiers in Biomedical Technologies, 2013 (in press).