**Category abstract:** Vascular biology

**Title**

“**In vitro and in vivo study on the effects of inhibitors of angiogenesis in atherosclerosis**”

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**Background/objectives**

Atherosclerosis is a lipoprotein-driven disease that leads to plaque formation at specific sites of the arterial tree through intimal inflammation, necrosis, fibrosis and calcification. Atherosclerotic plaques, can be classified in two main types: stable and unstable. Unstable plaques often show plaque angiogenesis and are prone to rupture, promoting thrombus formation which can lead to a myocardial infarction, stroke, and sudden death.

We are part of an European consortium called Moglynet, which aim is to stop intra-plaque neo angiogenesis by blocking the glycolytic pathway, more specifically the PFKFB3 enzyme. In order to do that the consortium is going to synthetize anti-angiogenic compounds and then study their effects, first on a panel of in vitro tests and then in vivo using vein graft surgery on mice, and our research group is going to focus on this last part of the project.

In order to test the anti-angiogenic capacity of new compounds we first synthetized a positive control for our future testing. After that we optimized both the aortic ring assay as a physiological in vitro angiogenesis assay and the in vivo model of vein graft surgery.

**Methods/Results**

To have a reliable positive control for our future in vitro and in vivo tests, in collaboration with Kemotech company (Sardinia, Italy) we worked on the chemical synthesis of a molecule known as K5. This molecule is able to bind and inhibit FGF2, a growth factor that play a critical role in neovascularization. From the chemical synthesis we got 1.30 g of molecule, more than sufficient to perform our experiment

Aortic ring angiogenesis assay

To test the anti-angiogenic capacity of new compounds we optimized the aortic ring assay. We cut pieces (rings) of a mouse thoracic aorta and embedded them in a suitable matrix in a 96-well plate. After adding angiogenic or anti-angiogenic compounds we observed the neovessels outgrowth over a period of 5-12 days. After 7 days we took pictures of the ring using a light microscope and quantified and characterized the sprouts growing out of the rings. For this we developed a triple immunofluorescent staining on the aortic rings to detect Smooth Muscle Cells with α-SMC actin Ab, Endothelial Cells with CD31 Ab, Macrophages with MAC 3 Ab and nuclei with DAPI and analysed z-stacks pictures using a confocal microscope. Moreover, we developed two different methods of quantification using ImageJ platform, first to quantify the number of sprouts growing from the different rings, and secondly to quantify the percentage of each type of cell present in the sprouts by analysis of triple stained z-stacks.

Mouse vein graft model

Lastly we will perform in vivo analysis of plaque angiogenesis using the mouse vein graft model. We use this tricky and complex surgery on ApoE3 Leiden mice as an in vivo model of atherosclerosis. This procedure involves the interposition of the caval vein of a donor mouse in the carotid artery of a recipient mouse. The formation of the lesion can be seen 28 days after the surgery. Currently I am learning this microsurgical operation technique.

**Conclusions**

With this panel of in vitro and in vivo tests we are now ready to start the tests of the molecules that the other members of the Moglynet consortium will provide to us.