Evaluation of coronary stenosis in rat: Toward the development of a chronic hibernation model

K. Montet-Abou[1], J-L. Daire[1], J-P. Jacob[1], M. Jorge-Costa[2], D. R. Morel[2], and J-P. Vallee[1]

[1]Radiology, Geneva University Hospital and Faculty of Medicine, Geneva, Geneva, Switzerland, [2]Anesthesiological Investigation Unit, Geneva University Hospital, Geneva, Geneva, Switzerland

Introduction:

Myocardial hibernation is a reversible state following chronic ischemia. It has first been characterized on patients after revascularization of supposedly infarcted myocardium. Animal research began to develop models of hibernation on dogs and pigs, but no rodent models have really been developed, except for a very recent model of transgenic mouse (May D, PNAS 2008). So the aim of our research is to fullfill this gap to obtain a convenient hibernation model. The three main difficulties associated to this development are: creation of a chronic ischemia without inducing myocardial infarct, repeated evaluation of the cardiac function and the definition of immuno-histological criteria of hibernation. The aim of the present abstract is to develop and characterize a coronary stenosis model in rat with respect to myocardial hibernation.

Material and Methods:

Sprague-Dawley rats (n=28) were anesthetized with 3% isoflurane and underwent cardiac surgery. One group was sham rats. The left coronary artery was stenosed in three animal groups with a suture probe from 175 µm to 275 µm diameter. The stenosis was provoked by positionning the suture including the probe around the left coronary artery, and the probe was quickly removed after the artery ligation. Then, they were imaged with Magnetic Resonance Imaging (MRI) at 1.5-T (Intera, Philips) ten days after the stenosis to evaluate the cardiac function as well as the presence of myocardial infarct. Cine imaging was realized using a prospective ECG-triggered segmented turbo field echo (TFE) cine sequence, TR/TE 12/4.9 ms. Flip angle (FA) 30°, 288x288 matrix sampled on a Cartesian grid, 80 mm field of view (FOV) and 2 mm slice thickness yielding an in plane resolution of 280x280 µm. Eleven to twenty cardiac phases where acquired per R-R cycle depending on the heart rate. Two short axial plane, as well as 2 and 4 cavities views were acquired for each animal. Tag images were obtained by C-SPAMM tag preparation segmented cine fast field echo sequence, TR/TE 7.8/3.6 ms, tag spacing 1.25 mm, acquired voxel size 0.63x1.79x3 mm, FA 10°. Tagged MR images were analysed with Extrema Temporal Chaining (Jacob J et al. Lecture Notes in Computer Science 2006;Volume 4179/2006:897-908).

After the MRI session, rats were sacrificed for histological analysis.

Results:

Different degrees of stenosis were obtained using probes of various diameter as attested by its quantification using HE staining (Fig1). The response to a given degree of stenosis was quite variable (from infarction to nothing) (Fig.1) but based on MRI analysis of regional contraction and response to dobutamine stress classification was achieved (Fig.2). Presence of infarction was detected with gadolinium late enhancement and correlated with histological analysis. Hence groups were determined using tag and cine analysis, which permitted accurate estimation of the regional and global heart function. We could class them in four categories: normal, ischemic, biphasic (hibernating) and infarcted pattern. Analysis are completed with immuno/histological data: glycogen and lipids accumulate in higher amount in hibernating heart, troponin was disorganized in hibernating and ischemic regions, and in some regions α-fetal smooth actin was reexpressed (Fig.3).
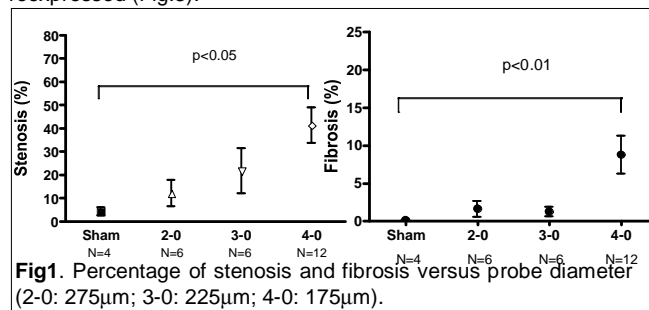

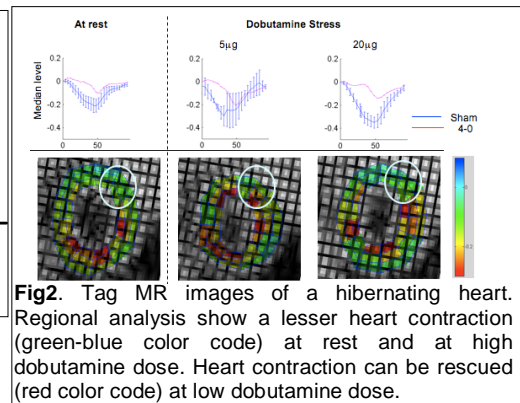**Fig1**. Percentage of stenosis and fibrosis versus probe diameter (2-0: 275µm; 3-0: 225µm; 4-0: 175µm).

Fig2. Tag MR images of a hibernating heart. Regional analysis show a lesser heart contraction (green-blue color code) at rest and at high dobutamine dose. Heart contraction can be rescued (red color code) at low dobutamine dose.
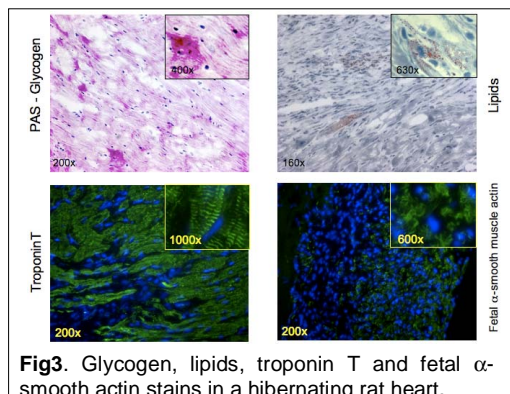

Fig3. Glycogen, lipids, troponin T and fetal α-smooth actin stains in a hibernating rat heart.

Conclusion:

Using MRI, we can detect regional dysfunction of myocardium contraction in a model of coronary stenosis. We were successfull to develop and characterize graded coronary stenosis in rats. For a given degree of stenosis we observed a variability in the myocardium response to the chronic ischemia.