

# EU-supported pre-clinical research with high translational value

Professor Dr Mariann Gyöngyösi from the Experimental and Clinical Invasive Cardiology Laboratory of the Medical University of Vienna discusses different animal models in the context of her group's research into treatments for cardiovascular diseases

Large animals still remain the best tool to investigate severe diseases, such as cardiovascular diseases,' Biondi-Zoccai G. 2013. One of the continuing aims of the pharmaceutical and medical device industries is the development of new and better pharmaceuticals and devices to help cure illnesses. Before applications of new medicines and medical devices reach humans, rigorous *in vitro* and *in vivo* experiments are performed to prove the safety of the new treatment mode. For *in vivo* testing, small (e.g. rodents) and large animals (e.g. pigs) are used, depending on the scientific and medical questions being asked, considering the '3R rules' (replace, reduce, refine) of animal experiments, and the veterinary ethical issues.

The appropriate animal model for a pre-clinical testing is chosen according to the drug or device being tested. For example, for toxicology and safety studies, small animals are usually recommended. For human size medical devices, such as vascular stents or implants (pacemaker, prostheses, artificial heart valves), large animals are the only choice to test the safety and, eventually, the efficacy of the device.

There are, however, several human cardiovascular diseases which can be modelled both in small and large animals, such as:

- Myocardial infarction
- Cardiac regenerative therapies
- Myocardial hypertrophy
- Diabetes, or
- Cardiomyopathies

The advantages of the small animal experiments is that they are cheaper by several order of magnitude, the numbers of experimental animals

can be much higher than that of large animals, and it is easy to investigate single genes by constructing knock-out animals. However, large animal models are mandatory for the preclinical testing of medicines and devices and other approaches with the requirement of high translational values because they yield functional data which are more reliable to human conditions as compared with rodent experiments. Additionally, large animal experiments allow a closer simulation of clinical settings regarding dosing and scalability and the delivery modes of medicines.

## A deciding factor

The size of animals is a deciding factor in many translational pre-clinical experiments. Imaging the cardiovascular system is useful to visualise the heart anatomy and function. For rodents, transthoracic echocardiography, micro-magnetic resonance imaging (micro-MRI), micro-computed tomography (micro-CT) or special catheterisation procedures can be performed, measuring limited physiological parameters, such as thickness of the ventricular wall in short-axis by M-mode view, and calculating fractional shortening of the left ventricle or three-dimensional parameters (such as left ventricular volumes) from that two-dimensional view.

To verify the infarct size in rodents, histological staining has proved to be the most appropriate method. The mouse echocardiography parameters are very small; for example, a normal end-diastolic volume (EDV) is 0.06-0.1 cm<sup>3</sup> – just a 2mm shift from the real dimension, which means a 0.008 cm<sup>3</sup> difference (13%) increase or decrease in EDV. To compare, the EDV of the heart of an 80kg healthy pig is approximately 80mL, much the same as the heart of a healthy man with a similar weight.

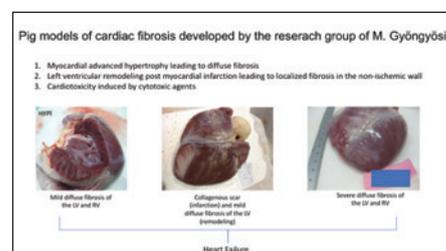


Fig. 1

In contrast to small animals, large animal imaging can be done with clinical cameras (e.g. CT or MRI), using the same projections and imaging procedure. Currently, an MRI with late enhancement imaging is the accepted standard for translational myocardial infarction processes, delivering comparable sized left ventricle and volumes as found in humans.

## Advantages and differences

The indisputable advantages of the large animal models in translational research are the similarities of the heart and circulation (patho)physiology between large animals and humans. The size, anatomy, and structure of the heart, vessels, and heart valves, as well as the circulatory physiology and pathophysiology of the pigs, are very similar to patients. Accordingly, to study the myocardial ischemia and infarction, ischemic cardiomyopathy and rhythm disturbances, cardiac regeneration and repair therapies, pigs are the most appropriate animals for translational research.

The robust difference between the small and large animal experiments regarding the translational value in cardiac regeneration trials are, among others:

- The different regeneration capacity of the heart of the species

- The open chest-chronic coronary occlusion in small animals
- The magnitude of doses of medicines for therapy
- The delivery mode of regenerative substances (intravenous or intraperitoneal or direct intramyocardial in rodents)

Chamuleau *et al.* published a meta-analysis of small and large animal trials involving cardiac regeneration.<sup>1</sup> This analysis revealed a publication bias in small animal trials, pointing out a robust overestimation of the results and efficacy of the cell-based cardiac regenerative therapies.

## Experience

To enhance the translational value of research in the field of medicines and medical devices, the research group of Professor Dr Mariann Gyöngyösi, at the Experimental and Clinical Invasive Cardiology Laboratory of the Department of Cardiology, Medical University of Vienna, have been performing large animal experiments for several years.

The two main focuses of her research are the implantation of new medical devices (coronary stents, other vascular stents, the use of drug-eluting balloons, introducers, occluders of atrial septal defect, patent foramen ovale or left atrial appendage, transcatheter valves), and myocardial ischemia-induced injury, infarction and cardiac regenerative and repair therapies and heart failure (see Fig. 1). The large animal experiments are performed in co-operation with the Large Animal Research Facility of University of Kaposvar/Medicopus, Hungary (see Fig. 2).

Among several academic and company-sponsored research projects and trials involving large animals and performed by the Gyöngyösi group in Vienna, three projects have received funding from the European Commission: LifeValve (finalised), Fibrotargets (finalised) and CresPace (on-going).

## The LifeValve project

The goal of the LifeValve project (Grant No. 242008) was to construct a tissue-engineered heart valve (TEHV), which can be implanted via cardiac catheter in children in pulmonary valve position without the need for open heart surgery.



Fig. 2

In contrast with the currently existing catheter-implantable artificial valves, the leaflet of this TEHV contains autologous stem cells, gained from the patient, aiming to inhibit the destruction of the valves by time and allowing for them to grow with the growing children.

The task of Gyöngyösi's group was to follow the fate of the stem cells seeded on the valve. The group transfected the mesenchymal stem cells with a positron emission tomography (PET) reporter gene and seeded the artificial valves with these transfected cells.

With the project's co-operation partners, the University of Debrecen and the Animal Lab at the University of Kaposvar, Hungary, the seeded artificial pulmonary valves were implanted in sheep via heart catheterisation. Following a pre-defined time interval, the animals were anaesthetised and a PET investigation combined with computed tomography (PET-CT) were performed. After administering a special PET tracer, the imaging showed an accumulation of the specific PET-tracer in the valves, confirming the survival of the mesenchymal stem cells on the valves, even after a period of six months following valve implantation. Histology also confirmed the presence of the mesenchymal stem cells, as well as the covering of the foreign body with autologous endothelial and smooth muscle cells. These experiments confirmed the theory put forward by this EU funded project.<sup>2</sup>

## The Fibrotargets project

The EU-funded Fibrotargets project (Grant No. 602904) was designed to search for pharmaceuticals and natural compounds which inhibit the development of myocardial fibrosis in different disease entities. While the consortium partners performed several small animal experiments, the Gyöngyösi group developed a new myocardial hypertrophy, fibrosis and secondary pulmonary hypertension model, based on the pre-clinical model of percutaneous artificial aortic isthmus stenosis.

The principle of this model is that the left ventricular muscle cells undergo severe hypertrophy due to the artificial stenosis of the aorta because the same blood amount should be streamed through a stenosis aortic wall. With time, the myocardium will undergo severe hypertrophy and fibrosis. For this reason, the group implanted metal stents in the descending aorta, immediately below the arcus aortae, in juvenile pigs. The stent was in-grown into the aortic wall, and prevented the normal widening of the aorta in the growing pig. After five-to-six months, the aorta wall becomes narrowed at the place of the stent, resulting in increased workload of the left heart chamber with consequent

hypertrophy and fibrosis with diastolic dysfunction and secondary pulmonary hypertonia. Through the slow increase of the stenotic effect, and pressure development, this model is more relevant for human conditions, as compared with so-called surgical aortic banding of the aorta in small or large animals with an abrupt increase in pressure and workload of the left ventricle.<sup>3</sup>

## The CresPace project

The on-going CresPace project (Grant No. 732170), has the goal of developing a new, more sophisticated pacemaker which can imitate the physiological heart rate variations more accurately in patients with heart electric conduction abnormalities. The task of the Gyöngyösi group is to test the newly constructed pacemaker in large animals and to verify the pacemaker-guided heart rhythm which is triggered by breathing and exercise-derived physiologic parameters.

Since large animal experiments require higher personal and material investments and costs, the financial support of the EU Commission is crucial for performing academic research with high human relevance. The expected societal, knowledge, and economic impact of these research projects will translate to human conditions, and so improve quality of life and reduce morbidity-associated hospitalisation and mortality of patients with cardiovascular diseases.

## References

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