Macro- and micromorphometric studies of the vascular structures from the Göttingen Minipig

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ABSTRACT

Porcine models have become increasingly popular in cardiovascular research, because physiological and anatomical features of the cardiovascular system from pigs including the coronary vascular system and the coronary collateral vessels are comparable to humans. The standard farm pig rapidly increases in body weight and size, potentially confounding serial measurements of cardiac function and morphology. In contrast Göttingen Minipigs have a characteristic growth curve that avoids the dramatic increase in weight in adulthood seen in farm pigs. The Göttingen Minipig is especially suitable for long-term studies because of its inherent small size and ease of handling, even at full maturity, which is reached at 2 y of age compared with 3 y for domestic pigs. However, there is still a need on detailed information about the macro- and microvascular characteristics of the vascular system from the Göttingen Minipigs.

The study was aimed to describe the macro- and microvascular characteristics of adult Göttingen Minipigs (n=18) by use of CT-imaging and histology over a time period of 4 month starting from 16 month of age up to 20 months. The animals showed no clinical symptoms of disease and were kept in-house at a light/dark rhythm of 12:12 under defined climatic conditions. The experiments were licensed by the regional authorities for health and social affairs (LaGeSo), Berlin. The study included the measurement of the length and of the luminal diameter of arteries and veins from the neck, thorax, abdomen, and limbs which are frequently used in experiments with pigs. In addition microscopical and histological parameters (luminal vessel diameter, thickness of the tunica externa, tunica media, and tunica interna) were studied on hematoxylin-eosin-stained sections of the blood vessel.

Key words: minipigs, vasculature, vessel wall

INTRODUCTION

Porcine models have become increasingly popular in cardiovascular research, because physiological and anatomical features of the cardiovascular system of pigs (including the coronary vascular system and the coronary collateral vessels) are quite comparable to humans [1, 2, 3]. The standard farm pig rapidly increases in body weight and size, potentially confounding serial measurements of cardiac function and morphology. In contrast, Göttingen Minipigs have a unique growth characteristic that avoids the dramatic weight and size increase seen in farm pigs even when they reach the adulthood [4, 5]. The Göttingen Minipig was developed in the early 1960s at the Institute of Animal Breeding and Genetics (University of Göttingen, Germany) to reduce space requirements and housing costs for preclinical porcine studies [6]. They were created by crossbreeding the Minnesota Minipig with Vietnamese potbelly and German Landrace pigs [7]. The Göttingen Minipig is especially suitable for long-term studies because of its easy handling and inherent small size even at its full maturity, which is reached in 2 years while it takes 3 years for domestic pigs [8]. However, more detailed information about the macro- and microvascular characteristics of the Göttingen Minipigs has to be elucidated to prove its scientific usefulness.

MATERIALS and METHODS

Animals, housing and care

In-vivo experiments approved by the regional office for health and social welfare (LaGeSo) of Berlin were performed at the Charité University Clinic, Campus Virchow (Berlin, Germany), Department of Experimental Medicine (certified by ISO 9001) using adult female pigs (n=18) from the Göttingen Minipig (Ellegaard, Denmark). At the beginning of the test the animals were 17 months old and had a body weight of 26.3 ± 1.8 kg. The animals were cared according to the guidelines of the European societies of laboratory animal sciences. Animals were housed as a group of 6, in an environmentally controlled room (12/12 light/dark-rhythm, 15-24 °C, 55±10 % relative humidity).

Anaesthesia

Prior to CT-Imaging and tissue sampling anaesthesia was performed. One night before anaesthesia the animals were fastened, but had free access to water. The minipigs were premedicated by intramuscular (i.m.) injection of 0.5 ml atropine (Atropinum sulfuricum, 1mg/ml, Eifelfango, Germany) and anesthetized by i.m. injection of ketamine (i.m., 27 mg/kg, UrsotaminTM, 100 mg/ml, Serumwerk Bernberg, Germany), xylazine (i.m., 3.5 mg/kg, RompunTM TS, 20 mg/ml, Bayer Vital, Germany) and 3 ml azaperone (i.m., Stresnil, 40 mg/ml, Janssen Animal Health, Germany). Additionally an electrolyte solution (Ionosteril, Fresenius, Germany, 1000 ml) was continuously infused intravenously (i.v.).

Computed tomography

The vascular structures of the pigs were studied by computed tomography (CT) analysis at an age of 17, 19 and 21 month according to table 1. The CT scan was done in prone position on a 64-slice scanner (LightSpeed 64 ; GE Medical Systems, Milwaukee, IL, USA). The scan protocol used contrast-enhancement with automatic intravenous injection of 80 ml nonionic iodinated contrast medium (XenetiX 350, Guerbet GmbH, Germany 350 mg/ml iodine) in each pig. The scan parameters were standardized (voltage 120 kV, maximal 500 mA with automatic mA-optimization at a noise index of 15, mean mA 490; collimated slice thickness: 64×0.625 mm; total detector width: 55 mm; rotation speed: 0.4 s; table feed per rotation: 55 mm) so the scan speed was approximately 3 s for 30 cm scan length in the z-axis. For volumetric assessment 1.25 mm images were reconstructed without overlapping. Basic image analysis was performed using Advantage Windows 4.2 (GE Medical Systems, Milwaukee, IL, USA) and AccuLite (AccuImage Diagnostics Corporation, South San Francisco, CA, USA).

Histology

Histological examination was done on 5 representative arteries (elastic arteries: Aorta thoracica, Aorta abdominalis, Arteria carotis communis; muscular arteries: A. femoralis, A. renalis) and 5 representative veins (Vena cava cranialis, V. cava caudalis, V. jugularis externa, V. renalis, V. femoralis). After embedding into paraffin, cross sections of these blood vessels with a thickness of 5 µm were made and stained using hematoxylin-eosin. Five slides per sample were prepared and each slide was evaluated at five different fields of view. The thickness of the tunica media and the thickness of the lamina endothelialis were determined using a transmitted light microscope (AxioSkope 2, Zeiss, Germany) and the imaging software AxioVision (Zeiss, Germany).

Statistics

Data are reported as mean value ± standard deviation for continuous variables or as selective frequency for categorical variables and analyzed by Student's t-test or the Chi square test. A p value of less than 0.05 was considered significant.

RESULTS

Body weight

The body weight increased from 26.3 ± 1.8 kg to 33.8 ± 4.7 kg (+ 28.5 %, during the time period between 17 and 21 month of age).

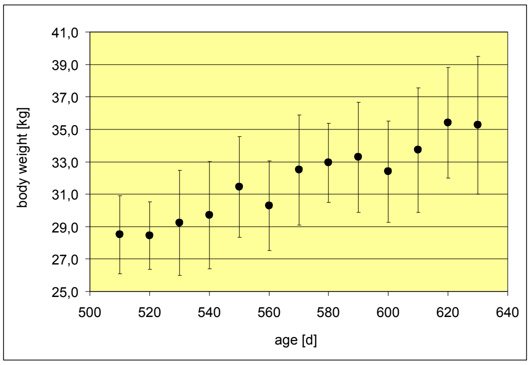

Figure 1: *Body weight of Göttingen Minipigs in the age range of 17 to 21 month; (mean value ± standard deviation, n = 18)*

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CT analysis

Ct analysis revealed that the luminal diameter and the length of the arteries and veins did not change during the time period between 17 and 21 month of age ($p<0.05$, figures 2).

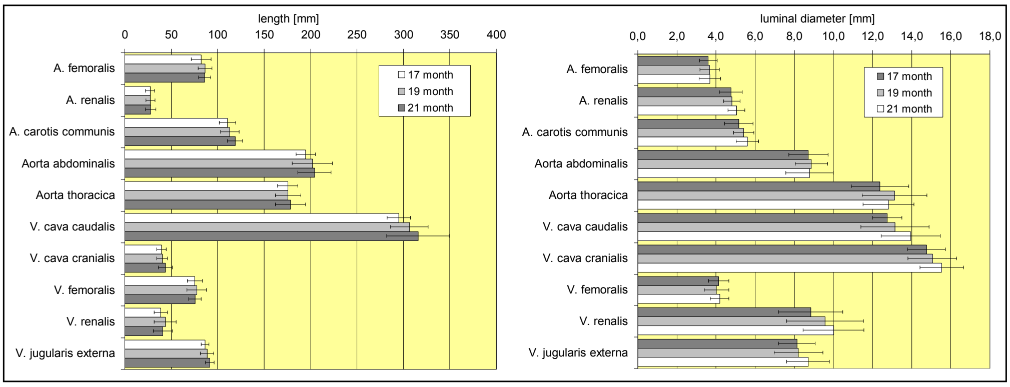

Figure 2: *Luminal diameter and length of veins and arteries of Göttingen Minipigs (during the time period between 17 and 21 month of age); CT-based data; (mean value ± standard deviation, n = 18)*

Histology

Figure 3 shows the thickness of the tunica media and of the lamina endothelialis from arteries and veins of the animals at an age 21 month. The thickness of the lamina endothelialis showed no significant difference between the veins and arteries.

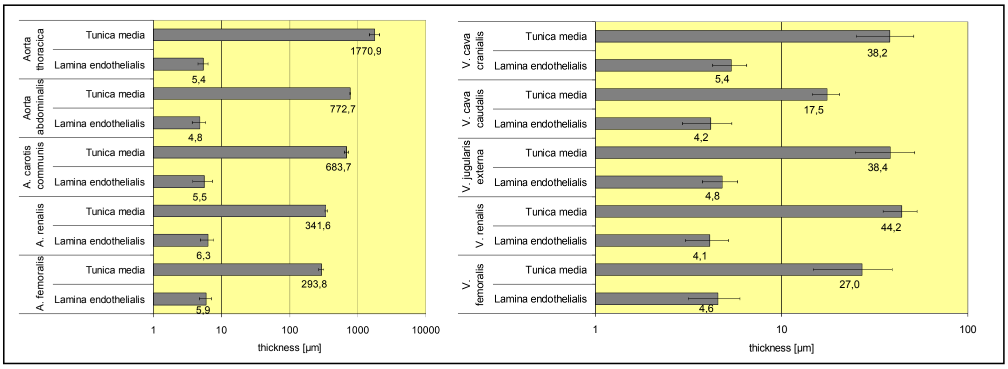

Figure 3: *Thickness of the tunica media and the lamina endothelialis of arteries and veins of 21 month old Göttingen Minipig; data based on histological examination; (mean value ± standard deviation, n = 3)*

DISCUSSION

In order to investigate the properties of implantable biomaterials a biological model is essential before establishing a clinical trial [9]. Therefore, the selection of the appropriate animal model is a major part of experimental work.

The study showed that the vasculature - luminal diameter and the length of the arteries and veins analyzed - remained constant over time in young adult Göttingen Minipigs despite of a significant increase of the body weight ($p<0.05$) over 4 months (from the 17. to the 21. month). This is in good agreement with recently published data showing that the diameters of the iliac bifurcation arteries of minipigs (Yucatan) did not change with increasing body weight [10].

In summary the results showed that the adult Göttingen Minipig proved to be a suited animal model for in-vivo studies which require long-term stable vascular macro- and micromorphometric parameters, such as implantation of stents, vein cuffs, heart or venous valves and vascular prostheses. The histomorphometric parameters (thickness of the tunica media and the lamina endothelialis) can provide helpful baseline data for developing implants.