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Research Article

Segmental and age differences in the elastin network, collagen, and smooth muscle phenotype in the tunica media of the porcine aorta

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ABSTRACT

The porcine aorta is often used in studies on morphology, pathology, transplantation surgery, vascular and endovascular surgery, and biomechanics of the large arteries. Using quantitative histology and stereology, we estimated the area fraction of elastin, collagen, alpha-smooth muscle actin, vimentin, and desmin within the tunica media in 123 tissue samples collected from five segments (thoracic ascending aorta; aortic arch; thoracic descending aorta; suprarenal abdominal aorta; and infrarenal abdominal aorta) of porcine aortae from growing domestic pigs ($n = 25$), ranging in age from 0 to 230 days. The descending thoracic aorta had the greatest elastin fraction, which decreased proximally toward the aortic arch as well as distally toward the abdominal aorta. Abdominal aortic segments had the highest fraction of actin, desmin, and vimentin positivity and all of these vascular smooth muscle markers were lower in the thoracic aortic segments. No quantitative differences were found when comparing the suprarenal abdominal segments with the infrarenal abdominal segments. The area fraction of actin within the media was comparable in all age groups and it was proportional to the postnatal growth. Thicker aortic segments had more elastin and collagen with fewer contractile cells. The collagen fraction decreased from ascending aorta and aortic arch toward the descending aorta. By revealing the variability of the quantitative composition of the porcine aorta, the results are suitable for planning experiments with the porcine aorta as a model, i.e. power test analyses and estimating the number of samples necessary to achieving a desirable level of precision. The complete primary morphometric data, in the form of continuous variables, are made publicly available for biomechanical modeling of site-dependent distensibility and compliance of the porcine aorta.

1. Introduction

The porcine aorta is often used in studies on morphology, pathology, transplantation surgery, vascular and endovascular surgery, and biomechanics of the large arteries. Although aged human aortae contain less elastin and more collagen and are therefore stiffer than the corresponding porcine aortic segments (Martin et al., 2011), there are no alternative large laboratory species. The pig aorta is currently the best described and most suitable animal model of the human aorta in terms of its caliber as well as gross and microscopic morphology or physiology.

Examples of studies using porcine aorta as a model for the human aorta comprise models of aortic coarctation (Hu et al., 2008), post-vagotomy remodeling (Sokolis et al., 2005), thoracic sympathectomy (Angouras et al., 2012), and models of abdominal aortic aneurysm (AAA) (Hynecek et al., 2007; Molacek et al., 2009; Houdek et al., 2013). Porcine models of AAA are used for the introduction of mesenchymal stem cells into aortic injury (Turnbull et al., 2011), simulation of ruptured AAA repair (Suk et al., 2012), testing of endovascular repair techniques (Lederman et al., 2014), and

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stent grafts (Saari et al., 2012). Worthley et al. (2000) showed that the porcine abdominal aorta has histopathological lesions that are comparable to human atherosclerosis.

1.1. The tunica media as a carrier of the mechanical properties of the aortic wall

As a typical elastic artery (Mayersbach, 1956), the tunica media of the aorta is a composite structure consisting of vascular smooth muscle cell (VSMC) layers that are periodically reinforced by an elastic network and collagen fiber bundles (Shadwick, 1999). The matrix fibers and the amorphous matrix are produced by and attached to the VSMC (Shadwick, 1999). The specific influence of these constituents on the mechanical behavior has been assessed by their selective removal from the vessel wall (Gundiah et al., 2007; Gundiah et al., 2009; Gundiah et al., 2013; Zou and Zhang, 2012; Zeinali-Davarani et al., 2013; also cf. Kochova et al., 2012). The contribution of elastin to the inflation response of the porcine thoracic aorta has been thoroughly mapped by Lillie et al. (2012). The tunica media of the porcine aorta seems to have similar elastic properties throughout its thickness (Stergiopulos et al., 2001; Gundiah et al., 2007). Using the isolated elastic network, advanced biomechanical models of porcine aortic elastin behavior have been developed (Watton et al., 2009; Lillie et al., 2010; Weisbecker et al., 2013). The application of elastin-specific magnetic resonance contrast agents allows for three-dimensional imaging (Makowski et al., 2012) at a lower resolution than conventional histology.

The inhomogeneous distribution of collagen fibers in the porcine aortic wall was recently reported by Sugita and Matsumoto (2013a), who detected higher collagen content in the dorsal and distal parts of the aorta than in the ventral and proximal wall regions. This finding might explain the localization of the predilection sites of aortic wall disruption, such as dissection and aneurisms, because its tensile strength depends on the intramural collagen fiber content and alignment (Kochova et al., 2012; Sugita and Matsumoto, 2013b).

VSMCs contain not only actin microfilaments and desmin intermediate filaments, they also contain vimentin intermediate filaments (Gabbiani et al., 1981; Fujimoto et al., 1987). VSMCs can modulate their phenotype from contractile (more actin and desmin) to synthesis (less actin and desmin, more vimentin, and higher proliferative activity) (Miyazaki et al., 2002; Owens et al., 2004; Boccardi et al., 2007). Actin, desmin, and vimentin can be detected in both porcine and human aortic samples using the same antibodies, their immunopositivity is robust and therefore easily to be quantified (Witter et al., 2010; Houdek et al., 2013; Eberlova et al., 2013). The Advanced models of the aortic wall are currently able to link the macroscopic mechanical behavior of the whole arteries with the cytoskeletal filaments of individual VSMCs (Unterberger et al., 2013).

1.2. Age-related and segmental differences in the porcine aorta

For experiments on the porcine aorta, animals of various ages are used, depending on the study design, experiment time points, post-operative follow-up, diet duration, etc. Most of the studies cited above analyze animals ranging in age between 0 days up to 6 months, depending also on the period of analysis. However, studies on postnatal ontogenesis of the porcine aorta composition are scarce, although changes in the tunica media composition described in people (e.g. Martin et al., 2011, Tsamis et al., 2013) might alert us to consider these developmental changes.

In general, the abdominal aorta is more accessible for vascular or endovascular surgery than the thoracic aorta; therefore, it has been established as a model of thoracic aortic dissection and in the application of vascular prostheses (Witter et al., 2010; Dziodzio et al., 2011; Okuno et al., 2012; Johnson et al., 2013). Using aorta segments as a model that differs from the natural site of the modeled disease might have drawbacks, especially if the aortic wall composition and properties differ between the target sites.

Anatomically, the porcine aorta consists of the ascending aorta, aortic arch, and descending aorta, which is subdivided into the aorta thoracica and aorta abdominalis. The latter is often divided further into the suprarenal and infrarenal abdominal aorta. The structural and mechanical properties of the porcine aorta vary as a function of distance from the heart. Davidson et al. (1985) suggested that the VSMC phenotype of the porcine aortic wall is distinct in different regions. Lillie and Gosline (2007) reported that the elastin meshwork in the thoracic aorta might become progressively anisotropic with increasing distance from the heart. Sugita and Matsumoto (2013a) found more collagen in the distal and dorsal aortic regions than in the proximal and ventral regions. Tremblay et al. (2010) reported heterogeneous orientation, density and contractile properties of aortic VSMCs within the ascending porcine aorta. Further segmental differences of the smooth muscle orientation along the entire porcine aorta have recently been reported (Tonar et al., 2015).

Histological and biomechanical differences between the aortic segments were mapped by Sokolis (2007) and Sokolis et al. (2008), who found a predominance of elastin over collagen in the proximal segments of aortic wall as well as a predominance of collagen over elastin in distal segments. Kim and Baek (2011) and Kim et al. (2013) reported a biomechanical variability among circumferential and longitudinal aortic regions, reporting the greatest elastic moduli in the posterior distal regions. However, systematic histological and histopathological studies, including of the whole porcine aorta, as in Sokolis et al. (2008), are still very rare in spite of the importance and number of papers on the porcine aorta. Contributing to the knowledge on segmental and age-related differences became the rationale for our study.

1.3. Study aims

The aim of our study was to assess the quantity of elastin, collagen, alpha smooth muscle actin, desmin, and vimentin using transversal histological sections of the porcine aorta and to compare these data between single aorta segments and age groups. The following null hypotheses were formulated and tested:

H_0(A): The area fraction of elastin, collagen, smooth muscle actin, vimentin, and desmin is the same in all proximodistal aortic segments of the same individuals when comparing the aortae of growing domestic pigs (age 0–230 days).

H_0(B): The area fraction of elastin, collagen, smooth muscle actin, vimentin, and desmin is the same for suckling piglets, weaners, and fattening pigs when comparing corresponding aortic segments.

H_0(C): The area fraction of elastin, collagen, smooth muscle actin, vimentin, and desmin does not correlate with the intima-media thickness or the wall thickness of the porcine aorta.

2. Materials and methods

2.1. Animals and specimen preparation

Whole aortae were collected from domestic pigs within a typical age range for experimental and model approaches (Molacek et al., 2009; Houdek et al., 2013; Witter et al., 2010). The animals (commercial fattening hybrids, $n = 25$; 12 males, 11 females, one castrated male, one without documented sex; age 0–230 days; weight 0.7–95 kg) were euthanized in a time frame comparable to other experiments (Ondrovics et al., 2013; Gabner et al., 2012; Worliczek et al., 2010). All of the animals were raised conventionally