Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 1148–1160
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Valve proteoglycan content and glycosaminoglycan fine structure are unique to microstructure, mechanical load and age: Relevance to an age-specific tissue-engineered heart valve

Elizabeth H. Stephens, Chia-Kai Chu, K. Jane Grande-Allen *

Department of Bioengineering, Rice University, P.O. Box 1892 – MS142, Houston, TX 77251-1892, USA

Received 13 December 2007; received in revised form 1 February 2008; accepted 20 March 2008
Available online 8 April 2008

Abstract

This study characterized valve proteoglycan and glycosaminoglycan composition during development and aging. This knowledge is important for the development of age-specific tissue-engineered heart valves as well as treatments for age-specific valvulopathies. Aortic valves and mitral valves from first–third trimester, 6-week, 6-month and 6-year-old pigs were examined using immunohistochemistry for versican, biglycan, decorin and hyaluronan, as well as elastin and fibrillin. The fine structure of glycosaminoglycans was examined by fluorophore-assisted carbohydrate electrophoresis. Decorin expression was strongest in the 6-year-old valves, particularly in the aortic valve spongiosa. The quantity of iduronate was also highest in the 6-year-old valves. The central tensile-loading region of the anterior mitral leaflet demonstrated reduced glycosaminoglycan content, chain length and hydration and a larger fraction of 4-sulfated iduronate and lower fraction of 6-sulfation. With age, the anterior leaflet center showed a further increase in 4-sulfated iduronate and decrease in 6-sulfation. In contrast, the anterior leaflet free edge showed decreased iduronate and 4-sulfated glucuronate content with age. The young aortic valve was similar to the mitral valve free edge with a higher concentration of glycosaminoglycans and 6-rather than 4-sulfation, but aged to resemble the mitral anterior leaflet center, with an increase in 4-sulfated iduronate content and a decrease in the 6-sulfation fraction. Elastin and fibrillin often co-localized with the proteoglycans studied, but elastin co-localized most specifically with versican. In conclusion, composition and fine structure changes in valve proteoglycans and glycosaminoglycans with age are complex and distinct within valve type, histological layers and regions of different mechanical loading.

Keywords: Valve; Proteoglycans; Glycosaminoglycans; Development; Aging

1. Introduction

Proteoglycans (PGs) and glycosaminoglycans (GAGs) are critical to the function of numerous soft connective tissues, including heart valves, providing material properties such as viscoelasticity and resistance to compression and tension [1,2]. PGs and GAGs also play crucial roles in tissue differentiation, growth factor regulation and various pathologies [2]. During fetal development, PGs and GAGs are the predominant components of the nascent heart valves [3]. In the mature mitral valve (MV), the composition of PGs and GAGs has been shown to vary regionally based on the type of mechanical loading experienced by these tissues [4]. Altered proportions of selected PGs and GAGs are found in myxomatous MV disease [5], are probably responsible for the altered material properties of these swollen tissues [6], and are possibly involved in the disease pathogenesis. The loss of the GAG- and PG-rich spongiosa layer, which normally provides shear between the outer valve layers and thus enables complex valve leaflet movement [7], from porcine bioprosthetic aortic valves (AVs) is thought to be instrumental in the failure of these devices [8]. Overall, knowledge of the composition and distribution

* Corresponding author. Tel.: +1 713 348 3704; fax: +1 713 348 5877.
E-mail address: grande@rice.edu (K.J. Grande-Allen).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.03.014

of the various GAGs and PGs within heart valves appears to be essential for understanding and recapitulating the complex mechanics of the heart valve leaflets. It is unknown, however, how the composition and distribution of these PGs and GAGs change throughout development and aging, when the heart valves experience significant changes in mechanical loading and tissue differentiation. Because these PG and GAG changes are probably important to valve function, characterizing them would contribute to the understanding of age-specific valve pathologies as well as the design of an age-specific tissue-engineered heart valve (TEHV).

While a limited number of reports have described the GAGs found in valves [9–13], these largely have not considered the complex valvular microstructure, the effect of subject age and GAG fine structure, i.e., the abundance of variously sulfated disaccharides. Sulfation patterns on GAG chains are thought to be critical to GAG function and cellular signaling [14–16]. Although a previous study on GAGs in human MVs considered age [4], the age-range was limited to the adult years. Moreover, characterizations of PGs and GAGs during heart and heart valve development have been limited to specific PGs and signaling pathways [17,18]. Reports characterizing GAGs in the AV are even fewer and largely assess bioprosthetic valves and TEHVs [19–24].

Therefore, it was the aim of this study to characterize the composition and fine structure of GAGs and PGs during development and aging of porcine AVs and MVs. As described above, this work represents the first such analysis of the AV, and changes in GAG composition during development have not been studied in either the AV or MV. In this study, a novel combination of two approaches, immunohistochemistry (IHC) and fluorophore-assisted carbohydrate electrophoresis (FACE) was used to provide detailed information regarding layer and region-specific PG and GAG distribution as well as the quantity and fine structure of various GAGs in different regions of the valve. IHC was also used to co-localize the specific PGs and GAGs with other matrix components with which they are known to interact, such as fibrillin, elastin and transforming growth factor beta (TGF-β). Porcine valves, which commonly serve as an animal model for human heart valve biology and pathology [25], were used because they were available in large numbers at a wide range of ages. Considering the substantial regional differences in mechanical loading of the MV, the MV leaflets were separated into regions experiencing compression (leaflet free edge, MVF) or tension (anterior leaflet center, MVAC).

2. Materials and methods

2.1. General procedure

Porcine hearts were obtained within 24 h of death (Fisher Ham and Meat, Spring, TX, for 6-week- and 6-month-old pigs; Animal Technologies, Tyler, TX, for fetal and 6-year-old pigs). Hearts were maintained on ice until processing. The sample set consisted of MVs and AVs from first fetal trimester, second trimester, third trimester, 6-week-, 6-month- and 6-year-old pigs. Three to five valves from each age group were used for IHC, whereas 10–14 valves from each postnatal age group were used for FACE. One leaflet per AV was used, chosen randomly from the non-coronary, right coronary and left coronary leaflets; for the MV only the anterior leaflet was used. Tissues used in IHC were fixed overnight in 10% formalin before cross-sections of the leaflet were cut from the annulus to the free edge, embedded in paraffin and sectioned to 5 μm thickness. For the AV, cross-sections were taken slightly off center to avoid the nodule of Arantius.

2.2. Histology and histochemistry

Each sample was stained histologically with Movat pentachrome to demonstrate the overall distribution of collagen (saffron yellow), elastin (hematoxylin black), and PGs/GAGs (alcian blue) within the valves. This staining enabled the identification of the different leaflet layers [26]. Histochemistry was performed to demonstrate the PGs versican (clone 2B1, Associates of Cape Cod, Falmouth, MA), decorin (LF-122, gift from Dr Larry Fisher, NIH), and biglycan (LF-104, gift from Dr Larry Fisher), since these are the predominant extracellular PGs found in valves [4], as well as the GAG hyaluronan (HA) using the biotinylated HA binding protein (Associates of Cape Cod). Sections were also stained using antibodies against the extracellular matrix components elastin and fibrillin (both from Abcam, Cambridge, MA), as well as the growth factor TGF-β (Biovision, Mountain View, CA), since those proteins are known to interact with versican, decorin and biglycan [1,27–29]. Because the binding site of the PG antibodies is on the core protein obscured by GAG chains, an enzymatic digestion of the GAG chains was used (200 mU ml^{-1} chondroitinase ABC (Associates of Cape Cod) 1 h, 37 °C) before placement of the primary antibody. A citrate-buffer-based antigen retrieval (30 min, 80 °C) was used in the staining of elastin, fibrillin and TGF-β. IHC samples were graded on a scale of 0–4 to evaluate marker intensity and delineation (marker contrast between valve layers) in each histological layer (ventricularis, atrialis (MV only), spongiosa and fibrosa). Quantification was based on a grading rubric for each characteristic (Fig. 1). Overall PG intensity was assessed using the combination marker "tPG," which was the sum of versican, decorin and biglycan staining for a given region/layer. The term "inflow layer" was defined to be the atrialis of the MV and ventricularis of the AV. A corresponding "outflow layer" was not compared between AV and MV because of their inherent differences in composition. For each of the 3–5 samples per age group, 1–3 sections were stained with a given antibody. Replicates, whether within the same IHC batch or in different IHC batches, were averaged. The quantification was performed twice, once by an unblinded

ID	Title	Pages
1547	Valve proteoglycan content and glycosaminoglycan fine structure are unique to microstructure, mechanical load and age: Relevance to an age-specific tissue-engineered heart valve	13