Does Erectile Tissue Angioarchitecture Modify with Aging? An Immunohistological and Morphometric Approach

Carla Costa, PhD, and Pedro Vendeira, MD, PhD

Laboratory for Molecular Cell Biology—University of Porto, Porto, Portugal

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ABSTRACT

Introduction. Erectile dysfunction is a common problem in aged men; however, which vascular cavernosal alterations occur with age progression remain unclarified.

Aim. Using cavernosal tissue from rats of various ages, we aimed to thoroughly assess erectile vascular-associated morphologic, immunohistological, and morphometric alterations during aging.

Methods. Male Wistar rats were divided according to age in groups of 2, 6, 12, 18, 24 months old (N = 5). Cavernosal tissue of all groups was collected and processed for morphologic evaluation, immunodetection of α-smooth muscle actin and von Willebrand factor and morphometric quantification of vascular and smooth muscle cell (SMC) areas.

Main Outcome Measures. The morphometric assessment of age-related alterations in cavernosal vascular and SMCs using the ImageJ image-processing program.

Results. Morphologic and immunohistological evaluation showed a similar structure of erectile tissue among all age groups, divided in two cavernosal bodies containing numerous sinusoidal vascular spaces surrounded by SMCs. Additionally, we observed a reduction of SMC content and an increase in the caliber of vascular spaces, with aging. This was confirmed by the morphometric quantification of the vascular and SMC areas (mean area $\times 10^3$ µm^2 $\pm \times 10^3$ standard error). Two-month-old animals had a mean vascular area of 4.21 ± 0.51, approximately 3.5-fold less than the 6-month-old group. The differences increased when comparing the youngest groups with the 12-, 18-, and 24-month-old animals, with mean measurements of 18.99 ± 1.91, 25.23 ± 2.76, and 26.34 ± 2.97. Conversely, SMC areas progressively decreased between 2- and 6-month-old animals, from 6.75 ± 0.90 to 6.38 ± 1.24. The elderly 12-, 18-, and 24-month-old groups presented an approximated 1.5-fold reduction on SMCs area, showed by the respective measurements of 4.11 ± 0.50, 4.01 ± 0.35, and 4.02 ± 0.44.

Conclusions. We demonstrated that cavernosal angioarchitecture was modified with aging. The decrease in SMCs and the considerable enlargement of vascular lumens may limit the basic function of penile vascular tree in the elderly. **Costa C, and Vendeira P. Does erectile tissue angioarchitecture modify with aging? An immunohistological and morphometric approach. J Sex Med 2008;5:833–840.**

Key Words. Erectile Dysfunction; Aging; Angioarchitecture; Cavernous Tissue

Introduction

Erectile dysfunction (ED) is defined as the constant inability to attain and maintain a penile erection sufficient for a satisfactory sexual performance. ED presents a high prevalence in the population, affecting almost 152 million men around the world [1]. Hypertension, hypercholesterolemia, and diabetes are known risk factors for the occurrence of ED [2]. In addition, it has been shown that the prevalence and severity of ED increases with the advance of age [3,4]. Several epidemiologic studies have shown an association between aging and male ED, demonstrating that the percentage of potent men decreases from 60% to 33% between 40 and 70 years of age [5–7]. Vasculogenic ED is recognized as the major etiology and refers to the impairment of the vascular perfusion to the corpora cavernosa, a process dependent on penile tissue vascular components,

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namely cavernosal endothelial and smooth muscle cells (SMCs). Penile erection is a neurovascular event, which requires profound corporal and helicine artery smooth muscle relaxation to allow blood flow to increase three to four times its normal level [8]. This allows the engorgement of the lacunar spaces, which causes the passive compression of the subtunical veins, thus producing penile rigidity. An adequate arterial blood supply is necessary for erectile function in order to fill the lacunar spaces, increase the cavernosal pressure, and activate the veno-occlusive mechanism [9]. Given the need for stringent control of blood flow during this process, any vascular insufficiency will drastically suppress erectile capability, translating into arterial flow and cavernosal smooth muscle relaxation impairment, fibrosis, and veno-occlusive dysfunction [10,11]. The integrity of the cavernosal endothelium is then crucial for the regulation of the vascular and muscular tone during penile tumescence, and any modification on the penile endothelial bed may contribute to pathophysiologic mechanisms involved in ED [12–14]. It has been reported that the process of aging may affect the function of corporal SMCs, being associated with alterations in the cavernosal expandability and subsequent veno-occlusive dysfunction [15,16]. Aging may also affect the structural and functional properties of the arterial wall, leading to vascular dysfunctions [17]. However, which vascular-associated changes occur in the erectile tissue with aging have not been thoroughly addressed. Therefore, we intended to characterize and evaluate alterations in the morphologic structure and organization pattern of the vascular endothelial component of the cavernosal tissue during aging.

Methods

Ethics

All the experimental procedures complied with the national regulations for the Care and Use of Laboratory Animals and were approved by the local ethics committee.

Animals

Male Wistar rats were raised at the Institute for Molecular and Cell Biology of the University of Porto, housed in cages and maintained in a 12-hour day/light cycle with free access to food and tap water. The animals were divided according to their age into five experimental groups (N = 5 in each animal group): Group I = 2 months old; Group II = 6 months old; Group III = 12 months old; Group IV = 18 months old; and Group V = 24 months old. Animals from the various groups were euthanized by decapitation, and their penises were excised. One-half of the penises were fixed in 10% buffered formaldehyde and embedded in paraffin, whereas the other half were frozen in an optimal cutting temperature embedding media and stored at −80°C. Cross paraffin sections and cryosections of equivalent penile tissue regions, 5- and 10-μm thick, respectively, were placed in 0.1% poly-L-lysine (Sigma-Aldrich, St. Louis, MO, USA) covered slides and used for the hematoxylin and eosin (H&E) morphologic evaluation and further immunohistological and morphometric analysis.

Dual Immunolabeling Detection of Cavernosal Smooth Muscle and Endothelial Cells

The presence of cavernosal SMCs and vascular endothelium was assessed by double immunofluorescence, using specific antibodies for α-smooth muscle actin (α-SMA) and the vascular glycoprotein von Willebrand factor (vWF). Briefly, rat penile tissue transversal cryosections were rehydrated for 15 minutes in phosphate buffered solution (PBS), followed by nonspecific binding inhibition with a blocking solution of 1% bovine serum albumin (BSA) in PBS, for 1 hour at room temperature. Then, sections were incubated overnight at 4°C in a humid chamber with a mixture of the primary antibodies diluted in PBS containing 1% BSA. A cross-reactive mouse antihuman α-SMA monoclonal antibody (dilution 1:500; clone ASM, Chemicon International, Harrow, UK) and a polyclonal cross-reactive rabbit antihuman vWF antibody (dilution 1:200; AB7356; Chemicon International) were used. In the following day, the sections were washed three times for 5 minutes each with PBS containing 0.1% Triton-X. Next, the slides were incubated for 1 hour with the following secondary antibodies diluted in PBS: a goat anti-mouse conjugated with a red fluorocrome (dilution 1:1000; Alexa 568, Lot 48029A, Molecular Probes, Invitrogen, Carlsbad, CA, USA) and a donkey anti-rabbit antibody conjugated with a green fluorocrome (dilution 1:1000; Alexa 488, Lot 38479A, Molecular Probes). Negative controls were performed in adjacent tissue sections, by replacing the primary antibodies with PBS containing 1% BSA. The sections were thoroughly rinsed for 20 minutes in PBS with 0.1% Triton-X, followed by nuclei counterstaining with 4′,6-diamidino-2-phenylindole (Invitrogen, Molecular Probes). Immunolabeled sections were then

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