ORIGINAL RESEARCH—BASIC SCIENCE

Effect of Aging and Cardiovascular Risk Factors on Receptor Tie1 Expression in Human Erectile Tissue

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ABSTRACT

Introduction. Erectile dysfunction is highly prevalent in patients with advanced age or cardiovascular disease risk factors (CVDRFs). These conditions interfere on expression of vascular growth factors and respective receptors causing disturbance in endothelial function.

Aim. This study aims to assess the effect of aging and CVDRF on the expression of tyrosine kinase with immunoglobulin-like and EGF-like domains (Tie) 1 in human corpus cavernosum (CC).

Methods. CC fragments obtained from programmed surgeries or organ donors were divided into three groups: young, healthy aged, and aged with CVDRF. Angiopoietin (Ang) 1, Ang2, Tie1, and Tie2 mRNA and protein levels were assessed by real-time polymerase chain reaction and Western blotting, respectively. Dual-immunolabeling of Tie1 with specific markers of endothelium and smooth muscle and Ang1 and Ang2 was performed.

Main Outcome Measures. To characterize the expression of Tie1 in human CC and elucidate its potential inhibitory effect in Ang–Tie2 system.

Results. Analysis of mRNAs demonstrated a decrease in Tie1 expression in CVDRF individuals compared with aged or young healthy individuals. No variation for Tie2, Ang1, or Ang2 expression was observed among the studied groups. In all analyzed CC fragments, a 125 kDa band, Tie1, was detected. This protein presented a significant age-related decrease, specially in individuals with CVDRF. Immunofluorescence study revealed Tie1 expression in the endothelium of samples of all experimental groups.

Conclusions. Employing different methodological approaches, we show for the first time that Tie1 is expressed in human CC endothelium, and its level of expression diminishes in aged individuals, particularly those with CVDRF. This finding reinforces the view that delivery of Ang1 to the CC of erectile dysfunction affected CVDRF patients is able to activate a beneficial Tie2 response. **Fonseca J, Tomada N, Magalhães A, Rodrigues AR, Gouveia AM, and Neves D. Effect of aging and cardiovascular risk factors on receptor Tie1 expression in human erectile tissue. J Sex Med 2015;12:876–886.**

Key Words. Aging; Angiopoietins; Cardiovascular Disease Risk Factors; Erectile Dysfunction; Human Corpus Cavernosum; Tie1

Introduction

Erectile dysfunction (ED) is frequently caused by cavernosal artery disease, failure of sinusoidal relaxation mechanisms, or veno-occlusive insufficiency that lead to ED of vasculogenic origin [1]. The main risk factors for ED of vasculogenic origin are shared with cardiovascular disease (CVD) and beyond aging, include hypertension, diabetes, obesity, and dyslipidemia that cluster in the metabolic syndrome [2,3]. Considering that patients with CVD or metabolic

syndrome (that associates with hyperglycemia and nonalcoholic fatty liver disease), frequently complain of ED, it is currently accepted the common background for these conditions [4,5]. In fact, ED was recently recognized as antedating cardiovascular events [1,6].

The endothelium that surrounds the lacunar spaces in the corpus cavernosum (CC) is crucial for the local vascular homeostasis and, in particular, plays a critical role in the regulation of vasomotor balance and blood flow. In fact, endothelium-derived nitric oxide (NO) pathway is the main intervenient in the cavernous smooth muscle fibers relaxation [7,8], thus promoting erection achievement and maintenance [9]. In contrast, endothelium integrity loss leads to NO-dependent vasodilatation impairment, a condition named endothelial dysfunction, that strongly compromises erectile function [10]. Indeed, ED was considered equivalent to endothelial dysfunction [11].

Although molecular mechanisms underlying ED remain largely unveiled, several lines of evidence show that local bioavailability of vascular growth factors intervene in the CC endothelial function and vascular integrity maintenance [12,13]. Among them is the vascular endothelial growth factor (VEGF) found to be downregulated in the CC of aged human and rat, thus contributing to ED [12,14]. This is a major change because VEGF is the main angiogenic factor that promotes endothelial cell proliferation and survival [15] and regulates NO synthesis [16]. Moreover, VEGF and its downstream signaling events are not the only contributors to vasculature protection, because other vascular growth factors as angiopoietins (Ang) can interplay with VEGF and modulate its functions [13].

The most studied angiopoietins (Ang1 and Ang2) are ligands of the "tyrosine kinase with immunoglobulin and EGF homology domain-2" (Tie2) receptor, expressed constitutively and ubiquitously throughout the vascular endothelium [17,18].

Ang1 is produced by peri-endothelial cells and mediates endothelial survival, vascular stabilization, and anti-inflammatory response after binding to Tie2 receptor. On the other hand, Ang2 is expressed by endothelial cells and presents a context-dependent activity. In fact, it induces vascular permeability and destabilization by competing with Ang1 for Tie2 engagement in the absence of VEGF [19], but when VEGF is available, it acts as a partial agonist, facilitating angiogenesis. In brief, when stimulated by Ang1, Tie2 receptors rearrange *in trans* complexes at endothelial intercellular junctions, promoting cell adhesion and survival, mainly via Akt kinase activation. In contrast, Ang2 stimulation of endothelial cells results in a weaker activation of Tie2 in cellular junctions, and in some conditions, it acts as an antagonist to inhibit the more robust Ang1-dependent Tie2 activation, promoting endothelial cell detachment from the underlying basal membrane [19,20].

Similarly to VEGF activation of its specific receptor 2 (VEGFR2), Ang–Tie2 system has already been proposed to be implicated in the regulation of endothelial function in the CC of both rat and humans [9,13].

An additional member of Tie receptors family, Tie1, was previously identified by us in the endothelium in CC of human origin employing immunofluorescence [21]. It is still considered an "orphan receptor," and its role in Ang-mediated signaling remains poorly understood. However, recent reports suggest that Tie1 could exert an inhibitory role in Ang–Tie2 activation, by means of a Tie family of receptors interaction prior to ligand engagement. Therefore, in endothelial cells expressing both Tie1 and Tie2, heterotypic complexes formation would inhibit Tie2 homodimer clustering and further activation by Ang1 or Ang2. Binding of Ang1 to Tie1–Tie2 complexes promotes heterodimer dissociation, Tie2 clustering and signaling initiation [17]. Interestingly, another study evidenced the VEGF intervention in Ang–Tie system regulation, by inducing proteolytic cleavage of Tie1 and further Tie2 activation [22].

In an attempt to clarify the role of Tie1 in Ang–Tie system regulation in the human CC and to elucidate its implication in vasculogenic ED onset, we proceeded to the angiogenic system main components assessment. To achieve this goal, the expression of Ang1, Ang2, Tie1, and Tie2 was analyzed by real-time polymerase chain reaction (PCR) and by Western blotting in samples of CC of individuals divided in groups according to the risk of developing ED.

Materials and Methods

Penile Tissue Collection and Processing

The CC fragments were removed from patients submitted to planned surgeries, after informed consent, and from organ donors, dissected simultaneously with organ harvesting for transplant program. The study design was authorized and approved by the local hospital and university ethics committees. The samples were divided into three