ORIGINAL RESEARCH

Effects of Aging and Cardiovascular Disease Risk Factors on the Expression of Sirtuins in the Human Corpus Cavernosum

Margarida Freitas, MD,*† Adriana R. Rodrigues, PhD,*† Nuno Tomada, MD, PhD,‡ João Fonseca, MD,*† Alexandre Magalhães, PhD,§ Alexandra M. Gouveia, PhD,*†¶ and Delminda Neves, PhD*†

*Department of Experimental Biology, Faculty of Medicine of Universidade do Porto, Porto, Portugal; †Instituto de Investigação e Inovação em Saúde, Institute for Molecular and Cell Biology (IBMC) of Universidade do Porto, Porto, Portugal; ‡Department of Urology, Central Hospital of S. João, Porto, Portugal; §REQUIMTE/Department of Chemistry & Biochemistry, Faculty of Sciences of Universidade do Porto, Porto, Portugal; ¶Faculty of Nutrition and Food Sciences, Universidade do Porto, Porto, Portugal

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ABSTRACT

Introduction. Sirtuin (SIRT)1 was recently identified in human corpus cavernosum (CC). We hypothesized that other sirtuins could also be expressed in the CC. Expression of these enzymes in tissues is affected by aging, the main independent risk factor for erectile dysfunction besides other cardiovascular disease risk factors (CVDRF), such as diabetes or obesity.

Aim. The aim of this study was to characterize the expression of SIRT1-3 and SIRT5–7 in human CC relatively to age and CVDRF.

Methods. Samples of CC collected from patients submitted to programmed surgeries or organ donors were divided in three groups according to age and presence of CVDRF. Expression of SIRT1–3 and SIRT5–7 mRNAs was assessed by real-time polymerase chain reaction. Cellular localization and semi-quantification of sirtuins proteins were performed by immunofluorescence and Western blotting (WB), respectively. Nuclear factor kappa B (NFkB)-p65, inducible (iNOS) and endothelial nitric oxide synthase (eNOS) levels were also assayed by WB.

Main Outcome Measures. The main outcome measure was to characterize the expression of SIRT1–3 and SIRT5–7 in human CC.

Results. SIRT1–3 and SIRT5–7 mRNAs were detected in all individuals, without statistical differences among groups, excepting SIRT7 that decreased four times in aged groups relatively to young ($P = 0.013$). WB analysis demonstrated that aged individuals with CVDRF presented higher levels of SIRT7 protein relatively to young ($P = 0.0495$) and lower levels of SIRT3 protein relatively to healthy aged ($P = 0.0077$). Expression of NFkB-p65 and iNOS were higher in aged than in young individuals ($P = 0.0185$; $P = 0.004$, respectively). No differences in other sirtuins or total eNOS were seen among groups although phospho eNOS Ser^{1177} levels decreased in groups of aged men relatively to young ($P = 0.0043$; $P = 0.0099$).

Conclusions. Our results demonstrate for the first time expression of SIRT2–3 and SIRT5–7 in the human CC. Aged individuals with CVDRF presented an increase in SIRT7 protein levels and a decrease in mitochondrial SIRT3. This finding suggests that CVDRF induces the loss of antioxidant defense mechanisms leading to endothelial injury.

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Key Words. Aging; Cardiovascular Disease Risk Factors; Human Corpus Cavernosum; SIRT1; SIRT2; SIRT3; SIRT5; SIRT6; SIRT7

Introduction

Aging strongly contributes to the development of endothelial dysfunction, characterized by a progressive decay in endothelium-dependent arterial vasodilatation [1,2] and diseases subsequent to endothelial injury, such as atherosclerosis, erectile dysfunction (ED) and cardiovascular disease (CVD). ED is predominant not only in the elderly, but also in patients with metabolic syndrome (MetS), which is an assembly of cardiovascular and metabolic risk factors such as visceral adiposity, insulin resistance/diabetes, high blood pressure, and dyslipidemia [3–5], being considered an early manifestation of further cardiovascular events [6]. Endothelium-derived nitric oxide (NO) pathway is the main intervenient in the cavernous smooth muscle fibers relaxation, fostering the achievement and maintenance of an erection [7,8]. The endothelial dysfunction-linked ED can be explained by decrease in expression and/or activity of endothelial NO synthase (eNOS) [9–14]. Akt/PKB-mediated phosphorylation at serine 1,177 is the main activating pathway of eNOS. Besides eNOS, other isoenzymes catalyse production of NO from L-arginine, including calcium–calmodulin-controlled neuronal NOS (nNOS), and the inducible isoform, iNOS that produces high levels of NO with potential oxidative cytotoxic effects. With age, the proportion of iNOS expression relative to eNOS tends to increase, which exhausts the substrates of the enzymes and favors oxidative conditions [15–18].

Additional activating mechanisms of eNOS, such as Sirtuin 1 (SIRT1)-mediated eNOS deacetylation had been recently described. SIRT1 is the best studied of the seven mammalian isoenzymes SIRT1–7, homologs of Silent mating-type Information Regulator (Sir)2 of yeast that exert NAD-dependent deacetylase and ADP-ribosylation activities [19]. The expression of each member of sirtuin family presents organelle and tissue-specificity. As well, the expression of each SIRT might change with aging or in dependence of specific metabolic conditions. SIRT1 is known to play important roles in endothelial cell function through eNOS activation, which inhibit vascular cell senescence and improve endothelial or even neuronal functions [20–22]. SIRT1 expression was demonstrated in the cytoplasm of smooth muscle cells (SMC) in the corpus cavernosum (CC) of rat and human origin [23]. It is, however, abundant in the nucleus where it suppresses transcription through histone deacetylation [24]. SIRT2 is found mainly in the cytoplasm where intervenes in tubulin deacetylation but also shuttles to the nucleus acting as a histone deacetylase and in control of the cell cycle [25,26].

SIRT3, SIRT4 and SIRT5 are mitochondrial enzymes with mitochondrial-targeting sequences in their N-termini [27]. Mitochondrial sirtuins intervene in the regulation of the activity of metabolic enzymes and thus have crucial roles in the metabolic adaptation to dietary conditions, such as energy restriction and fasting. SIRT3 is the best characterized mitochondrial sirtuin, able to upregulate acetyl-CoA synthetase 2 activity through deacetylation [28] and to improve antioxidant defense system [29,30]. SIRT4 and SIRT5 participate in metabolism control and regulation of the urea cycle, respectively [31].

SIRT6 and SIRT7 are far less studied. SIRT6 is known to be active in the cell nucleus where it regulates histone acetylation status and DNA double-strand break repair presenting an important role in aging phenotype modulation [32,33]. Supporting this evidence, SIRT6 depletion results in premature cellular senescence and telomere dysfunction [34]. SIRT7 seems to be a positive regulator of RNA polymerase I-mediated transcription [35]. Recently, SIRT7-knockout mice were reported to exhibit signs of age-related changes, such as kyphosis, loss of subcutaneous fat, degenerative heart hypertrophy, and premature death [36].

To address the hypothesis that sirtuins could associate with the risk of developing ED, we analyzed the expression of SIRT1, SIRT2, SIRT3, SIRT5, SIRT6, SIRT7 by real-time polymerase chain reaction (PCR), Western blotting (WB) and immunofluorescence in samples of CC from individuals divided in groups according to age and CVD risk, which as far as we know has never been studied before.

Materials and Methods

Penile Tissue Collection and Processing

Samples of human CC were collected from white patients submitted to penile deviation or corporoplasty surgeries after informed consent or from organ donors, simultaneously with the organ harvesting for transplant program. The experimental protocol was approved by Faculty of Medicine of Universidade do Porto and Hospital S. João Ethics' Committee. CC samples were divided into three groups according to age and pre-existing risk factors for ED; Young (N = 10; 16–35