ORIGINAL RESEARCH—BASIC SCIENCE

Diminazene Protects Corpus Cavernosum Against Hypercholesterolemia-Induced Injury

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ABSTRACT

Introduction. Angiotensin-converting enzyme 2 (ACE2) is a key enzyme of the renin angiotensin system, which breaks down angiotensin II and forms angiotensin-(1–7). In erectile tissues, it has been documented that angiotensin II contributes to the development of erectile dysfunction (ED), while treatment with angiotensin-(1–7) improves penile erection. However, the expression and function of ACE2 in erectile tissues have never been investigated.
Aim. Here, we examined the expression of ACE2 in erectile tissues and its actions against hypercholesterolemia-induced corpus cavernosum (CC) injury.
Methods. Hypercholesterolemic apolipoprotein E knockout ($ApoE^{-/-}$) mice, a well-known model of ED, were treated with diminazene aceturate (DIZE), an ACE2 activator compound, or vehicle for 3 weeks. Reactive oxygen species (ROS), collagen content, and protein expression of ACE2, neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) subunits were evaluated in the penis of DIZE-treated and untreated $ApoE^{-/-}$ mice. Functional studies were performed in CC strips.
Main Outcome Measures. ACE2 expression and its role in modulating nitric oxide (NO)/ROS production and fibrosis within the CC of hypercholesterolemic mice were the main outcome measures.
Results. ACE2 was expressed in smooth muscle and endothelial cells of mouse CC. Interestingly, ACE2 was downregulated in penis of hypercholesterolemic mice with ED, suggesting a protective role of ACE2 on the CC homeostasis. In accordance with that, pharmacological ACE2 activation by DIZE treatment reduced ROS production and NADPH oxidase expression, and elevated nNOS and eNOS expression and NO bioavailability in the penis of $ApoE^{-/-}$ mice. Additionally, DIZE decreased collagen content within the CC. These beneficial actions of DIZE on the CC were not accompanied by improvements in atherosclerotic plaque size or serum lipid profile.
Conclusion. ACE2 is expressed in erectile tissue and its reduction is associated with hypercholesterolemia-induced ED. Additionally, treatment with DIZE improved hypercholesterolemia-induced CC injury, suggesting ACE2 as a potential target for treating ED. **Fraga-Silva RA, Costa-Fraga FP, Montecucco F, Sturny M, Faye Y, Mach F, Pelli G, Shenoy V, da Silva RF, Raizada MK, Santos RAS, and Stergiopulos N. Diminazene protects corpus cavernosum against hypercholesterolemia-induced injury. J Sex Med 2015;12:289–302.**

Key Words. Erectile Dysfunction; Hypercholesterolemia; Angiotensin-Converting Enzyme 2; Angiotensin II; Angiotensin-(1–7)

Introduction

The renin angiotensin system (RAS) is a key modulator of cardiovascular homeostasis [1–3]. Also, evidences indicate that RAS also plays an essential role in erectile function [4–10]. Besides endocrinal actions, the RAS is articulated within the cavernosal tissue and acts in a paracrine manner, modulating corpus cavernosum (CC) smooth muscle cell activity [4,8,10]. In fact, the physiological amount of angiotensin II (Ang II), the main RAS effector, produced in erectile tissues is significantly higher than those found in the systemic circulation [4], indicating an intense modulation of RAS in the erectile tissues. Within the CC, Ang II produces smooth muscle cell contraction and oxidative stress and decreases nitric oxide (NO) bioavailability [8,11,12]. Moreover, it was reported that Ang II levels in penile plasma were elevated in patients with erectile dysfunction (ED) [4], suggesting that the hyperactivity of this peptide is closely associated with the pathogenesis of ED.

Conversely, Ang-(1–7), known as a pivotal contraregulator of Ang II, appears to mediate penile erection [6,7,9]. Ang-(1–7) produces CC relaxation and NO release and potentiates erection by acting through Mas receptor [7]. This peptide is mainly formed by angiotensin-converting enzyme 2 (ACE2), which cleaves the C-terminal phenylalanine of Ang II, leading to Ang-(1–7) formation [13–15]. Thus, ACE2 is a key enzyme of the RAS that reduces Ang II and increases Ang-(1–7) bioavailability. However, the expression and function of this enzyme have never been investigated in erectile tissues.

Recently, a novel pharmacological strategy has been developed to target ACE2. Based on the crystal 3D structure of ACE2 and using a virtual screening strategy, about 140,000 small molecules (from a chemical library) were molecularly docked into structural pockets present in structures of ACE2 in different conformations [16]. Small molecules able to interact with a specific pocket of this enzyme were able to maintain a favorable conformation for optimal activity, consequently activating the enzyme [16]. It was documented that two compounds are able to activate ACE2, a xanthenone called XNT [16–19] and diminazene aceturate (DIZE) [20–22]. It has been shown that ACE2 activator compounds produce several beneficial cardiovascular effects [16–22]. For instance, DIZE attenuates pulmonary hypertension [20], reduces damages in cardiac [21] and stroke ischemia [22], and has other beneficial outcomes [23,24]; however, its action on erectile tissues has never been addressed.

In the present study, we aim to evaluate the expression of ACE2 in erectile tissue. Moreover, we sought to investigate if treatment with the novel ACE2 activator compound, DIZE, would protect the CC against injury induced by hypercholesterolemia.

Materials and Methods

Experimental Design

A well-established animal model of hypercholesterolemia-induced ED was used [25–27]. This ED model was chosen foremost because hypercholesterolemia is one of the most prevalent risk factors for the development of ED [28,29]. Moreover, the pathophysiological mechanism of hypercholesterolemic ED condition involves molecular pathways related to actions of the RAS on systemic vasculature and CC, such as modulation of oxidative stress, NO bioavailability, and matrix collagen deposition [28,30–33]. Apolipoprotein E knockout ($ApoE^{-/-}$) mice in a C57BL/6J background (N = 40) were obtained from Charles River Laboratories (Les Oncins, France). Animals at 15–20 weeks of age were randomly assigned in two groups: (i) treated with the ACE2 activator DIZE [20] and (ii) treated with vehicle (saline 0.9%). During the 11-week experimental period, the animals were fed with Western-type diet consisting of 15% (wt/wt) cocoa butter and 0.25% (wt/wt) cholesterol (Diet W, cat# 4021.06, abDiets, Woerden, Netherlands). The mice were administered with DIZE (15 mg/kg per day, provided by Prof. Raizada) or vehicle subcutaneously during the last 3 weeks. After experimental period, the animals were anesthetized (ketamine 100 mg/kg, xylazine 10 mg/kg) and blood samples were collected by cardiac puncture for serum extraction. Immediately following cardiac puncture, the penis was removed and snap-frozen in liquid nitrogen and stored at –80°C for protein measurements or frozen in cryoembedding medium for histological analysis. Some penises were excised and mounted in the isolated organ bath as described below. Age-matched C57BL/6J wild type mice (n = 20) fed with standard diet were used as additional controls. All animal study was approved by local ethics committee and Swiss authorities and is in accordance with the United States National Institutes of Health (NIH) guidelines.

Dosage of Serum Lipid Profile

Serum lipid profile was routinely measured on total blood and expressed as millimole per liter.