An Increased Arginase Activity Is Associated with Corpus Cavernosum Impairment Induced by Hypercholesterolemia

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ABSTRACT

Introduction. Hypercholesterolemia is a prevalent risk factor for the development of erectile dysfunction (ED), mostly due to an increase in oxidative stress and impaired nitric oxide (NO) bioavailability within the penis. Arginase is an enzyme that shares the common substrate L-arginine with NO synthase. Augmented arginase activity reduces NO production and is associated with ED development. However, the contribution of arginase hyperactivity in hypercholesterolemia-induced ED is unknown.

Aim. In the present study, we investigated the activity and role of arginase in the corpus cavernosum of hypercholesterolemic mice.

Methods. Apolipoprotein E (ApoE) gene-deleted mice fed with a Western-type diet for 11 weeks were treated with the selective arginase inhibitor, N-ω-Hydroxy-L-norarginine (NOHA), or vehicle (saline 0.9%) during the last 9 weeks. Arginase activity and expression were measured in penis protein extraction. Reactive oxygen species (ROS) content within the corpus cavernosum was measured by dihydroethidium staining. Functional in vitro studies were performed using cavernosal strips mounted in an isometric organ bath to evaluate NO production.

Main Outcome Measure. Arginase activity and its role in modulating NO and ROS production within the corpus cavernosum of hypercholesterolemic mice is the main outcome measure.

Results. Total arginase activity and arginase type II protein expression were increased in hypercholesterolemic mice compared with wild-type mice. The long-term treatment with NOHA normalized this alteration. Moreover, pharmacological arginase inhibition by NOHA attenuated the augmented ROS production within the corpus cavernosum of $ApoE^{-/-}$ mice, which increased the NO-dependent response in cavernosal strips.

Conclusion. These evidences indicate that arginase hyperactivity is associated with ED induced by hypercholesterolemia, suggesting that this enzyme is a potential target for treating ED. **Fraga-Silva RA, Costa-Fraga FP, Faye Y, Sturny M, Santos RAS, da Silva RF, and Stergiopulos N. An increased arginase activity is associated with corpus cavernosum impairment induced by hypercholesterolemia. J Sex Med 2014;11:1173–1181.**

Key Words. Erectile Dysfunction; Arginase; L-arginine; Nitric Oxide; Oxidative Stress; Hypercholesterolemia

Introduction

Erectile dysfunction (ED) is a common, multicausal disorder associated with aging, smoking, and diverse pathogenic conditions, such as hypertension, hypercholesterolemia, and hyperglycemia [1,2]. The major mechanism responsible for ED is an increase in the tone and/or contractility of the smooth muscle within the corpus cavernosum and penile arteries [3], which is mostly caused by diminished production and function of nitric oxide (NO) and augmented production of vasoconstrictors and reactive oxygen species (ROS) [4–6].

Hypercholesterolemia is one of the most prevalent risk factor for the development of ED [2,5].

This condition leads to atherosclerosis development and produces various functional alterations in the vasculature [7,8]. In the penis, these vascular changes profoundly impair the arterial response to vasodilatory factors, mostly due to increasing oxidative stress and decreasing NO bioavailability [5,9,10]. In fact, studies have demonstrated that nicotinamide adenine dinucleotide phosphate oxidase, the main source of ROS generation, is upregulated in the corpus cavernosum of hypercholesterolemic animals [5,10,11], while nitric oxide synthase (NOS) levels and activity are reduced [5,11].

Arginase is an enzyme that catalyzes L-arginine to form L-ornithine and urea, sharing the common substrate L-arginine with NOS [12,13]. Therefore, arginase may regulate NO bioavailability due to substrate depletion in NO biosynthesis [12,14]. Regulation of arginase-mediated NO production has been confirmed in different cells types [13,15,16]. Moreover, the hyperactivity of arginase is involved in diverse pathological conditions, such as atherosclerosis [17,18], hypertension [19], diabetes [20], as well as ED [14,21–25]. For instance, previous studies have demonstrated that corpus cavernosum from diabetic patients with ED exhibits augmented arginase activity [26] and diminished NO synthesis, which was associated with reduced cavernosal relaxation. Furthermore, long-term oral administration of L-arginine reversed the decreased erectile function in aged rats [27], while chronic inhibition of arginase restored nitroso–redox balance and increased penile vascular compliance of aged rats [24]. However, the role of arginase in hypercholesterolemic-induced ED was not addressed. Here, we examine the activity and expression of arginase in the penis from apolipoprotein E gene-deleted ($ApoE^{-/-}$) mice, a well-known animal model of hypercholesterolemic-induced ED. Moreover, we investigated whether pharmacological arginase inhibition would improve the impairment induced by hypercholesterolemia in the corpus cavernosum.

Materials and Methods

Experimental Design

$ApoE^{-/-}$ mice in a C57BL/6J background (N = 40) were obtained from Charles River Laboratories (Les Oncins, France). Animals (15–20 weeks of age) were randomly assigned to receive N-ω-Hydroxy-L-norarginine (NOHA) or vehicle (saline 0.9%) treatment. During the 11-week experimental period, all animals were fed a Western-type diet consisting of 15% (wt/wt) cocoa butter and 0.25% (wt/wt) cholesterol (Diet W; abDiets, Woerden, Netherlands). During the last 9 weeks of this experimental period, mice were administered with NOHA (10 mg/kg, i.p., 5 days/week) or respective vehicle control. Age-matched wild-type mice were used as additional controls. The animals were euthanized with injection of ketamine 100 mg/kg and xylazine 10 mg/kg, and blood samples were collected by cardiac puncture for serum extraction. Immediately following cardiac puncture, the mice were perfused with phosphate-buffered solution (PBS), and the penis was removed and snap-frozen in liquid nitrogen and stored at –80°C for protein measurement or frozen in cryoembedding medium for histological analysis. Some penes were excised and mounted in the isolated organ bath to evaluate endothelial function. Total serum cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were routinely measured and expressed in mg/dL. All animal studies were approved by local ethics committee and Swiss authorities (Service Vétérinaire Cantonal Lausanne, authorization number 2026) and are in accordance with the Helsinki Declaration.

Arginase Activity

Total arginase enzyme activity in penis protein extraction from wild-type, untreated, and NOHA-treated $ApoE^{-/-}$ mice was assessed as previously described [28,29]. In brief, penile tissue were extracted and homogenized in ice-cold lysis buffer (50 mM Tris-HCl [pH: 7.4], 0.1 mM ethylenediamine tetraacetic acid, and 0.1 mM ethylene glycol tetraacetic acid) (1:4 [wt/vol]) in the presence of protease inhibitors (aprotinin, leupeptin, phenylmethanesulfonylfluorid). The homogenates were centrifuged for 10 minutes at 14,000 g, and the supernatants collected for enzymatic assays. Lysate aliquots (25 μL) were incubated with 25-μL reaction buffer (10 mM $MnCl_2$) and heated at 56°C for 10 minutes to activate arginase. Afterward, 50 μL of 0.5-mM L-arginine buffer was added and the mixtures were incubated at 37°C for 1 hour to hydrolyze L-arginine. The reaction was stopped by the addition of 400 μL of an acid buffer (0.72 M HCl), followed by the addition of 25 μL of 9% α-isonitrosopropiophenone (diluted in absolute ethanol), and heated at 100°C for 45 minutes for colorimetric determination. Samples were allowed to cool down at room tem-