High Cholesterol Feeding in C57/Blc6 Mice Alters Expression within The VEGF Receptor-Ligand Family in Corporal Tissue

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ABSTRACT

Introduction. Angiogenesis, the growth and proliferation of blood vessels from existing vascular structures, is mediated by many cytokine growth factors and receptors, among the most important are the vascular endothelial growth factor (VEGF) family.

Aim. Decreases in VEGF receptor signaling have been linked to abnormalities in vasoreactivity in corporal tissue, but it is unknown if alterations in the VEGF ligands and/or receptors contribute to this process.

Main Outcome Measures. We sought to determine changes in vasoreactivity and the expression of the family of VEGF ligands and receptors in corporal tissue with cholesterol feeding in C57BL6 mice.

Methods. Twenty-four mice (N = 8/group) were fed a normal diet (Group 1) or a 1.25% high cholesterol diet for 4 (Group 2) or 12 (Group 3) weeks. Isometric tension studies were performed on corporal strips and dose response curves were generated to evaluate endothelium-dependent and endothelium-independent vasoreactivities. Levels of VEGF-A, B, C, D, VEGF receptors (VEGFRs) were detected by PCR (polymerase chain reaction) and/or western blot/enzyme-linked immunosorbent assay (ELISA). Endothelial and smooth muscle cell contents were determined by immunohistochemistry.

Results. At 4 weeks there was a small but significant decrease in endothelium-dependent vasoreactivity. Both mRNA and protein levels of VEGFR-1 were decreased, while VEGF-B was increased in Group 2 vs. Group 1, with no change in VEGF-A or endothelial cell content. By 12 weeks, decreases in both endothelium-dependent and endothelium-independent vasoreactivity were evident with decrease in most VEGF ligands (except VEGF-B), receptors, and receptor signaling.

Conclusions. Cholesterol feeding in C57BL6 mice results in alterations in the VEGF receptor-ligand family that may initially serve to limit the degree of vascular injury but these adaptations fail with the continuation of cholesterol feeding. **Xie DH, Hazarika S, Andrich AJ, Padgett ME, Kontos CD, Donatucci CF, and Annex BH. High cholesterol feeding in C57/Blc6 mice alters expression within the VEGF receptor-ligand family in corporal tissue. J Sex Med 2008;5:1137–1148.**

Key Words. Nitric Oxide; Growth Factors; Endothelium; Corpus Cavernosa; VEGF; Angiogenesis

Introduction

Angiogenesis is defined as the growth and proliferation of blood vessles from existing vascular structures [1]. Vascular endothelial growth factor (VEGF) consists of a family of different genes, the major ones being VEGF-A, B, C, and D, which encode ligands for VEGF receptors (VEGFR) 1–3 [2–6]. VEGFR-1 and VEGFR-2 are closely related receptor tyrosine kinases, and while a clear role for both receptors can be found in embryonic development, VEGFR-2 is know to be a more active kinase for downstream protein targets and the major receptor in post-natal angiogenesis [7–10]. VEGFR3 plays a role that is generally regarded as limited to lymphangiogenesis. The affinity of the VEGF ligands for the receptors is not uniform. While VEGF-A will bind both

VEGFR1 and VEGFR2 (albeit with a greater affinity for R1), VEGF-B will almost exclusively bind VEGFR1, and VEGF-C and VEGF-D will bind almost exclusively VEGFR-2 and VEGFR-3 [5]. We recently showed that in the absence of a secondary injury, diabetes mellitus results in alterations in the VEGF recptor ligand family (specifically there were decreases in VEGFR1 and its splice variant) that optimized the potential for VEGF to bind to VEGFR-2, as opposed to VEGFR-1, and thus activate downstream signaling molecules [11].

It is well accepted that the risk factors for atherosclerosis cause vascular injury that has the potential to disrupt physiologic interactions among cells in vascular structure and this could also occur in corporal tissue [2,3,12–16]. Because hypercholesterolemia is the most important risk factor for the development of erectile dysfunction (ED) in humans, the purpose of our current study is to examine ex vivo vasoreactivity and then to test for changes in VEGF (vascular endothelial growth factor) receptor ligand family in corporal tissue of hypercholesterolemic C57BL6/J mice. In addition, we measured the downstream targets of VEGFR activation that include, directly, the phosphorylation and thus activation of Akt which in turn phosphorylates and activates endothelial nitric oxide synthase (eNOS), which has been shown to mediate VEGF-induced penile erection [4,5].

Materials and Methods

Mouse Model

Twenty-four wild-type C57BL6 mice were divided into three groups (N = 8/group). Group 1 was fed a regular/normal chow (Group 1 = Normal) and served as the control group. The other 16 were fed a 1.25% cholesterol (Research Diets, Inc, New Brunswick, NJ) diet for the final four (Group 2 = 4 weeks high cholesterol, HC) or 12 (Group 3 = 12 weeks HC) weeks of the study. All mice were 20–22 weeks old at study termination. All protocols were approved by the Duke University Animal Care and Use Committee.

Serum Lipid Analysis

The mice were fasted the night before harvest, and then their hearts were punctured the next morning under ketamine (50 mg/kg) and xylazine (5 mg/kg) anesthesia for blood collection. Serum was separated from the rest of the blood components by centrifugation at 3,000 rpm for 15 minutes. Total cholesterol levels were measured using an enzymatic in vitro test with Roche automated clinical chemistry analyzers (Roche Diagnostics GmbH, D-68298 Mannheim), as previously described [17].

Tissue Procurement, Histological Section Preparation, Protein, and RNA Isolation

At the study termination all of the mice were deeply anesthetized with ketamine and xylazine and a penectomy was performed followed by a careful dissection of the corpora cavernosa from the tunica albuginea. The corpora cavernosa was divided in manner such that each mouse randomly provided tissue for three of the four types of analyses (vasoreactivity, protein, mRNA, and histology). The mice were then sacrificed. For histology, tissue was cryoprotected and frozen sections (5 μm) were prepared. For protein studies using an enzyme immunoassay assay (ELISA), tissue samples were pulverized in liquid N_2 and homogenized in 0.1 M HCl (Assay Designs, Ann Arbor, MI) 3- to 5-x volume, using a Polytron (Brinkmann, Westbury, New York). For protein studies using western blot analysis, tissue samples were homogenized in a lysis buffer with protease and phosphotase inhibitors (10 mM Tris, pH 7.4, and 100 mM NaCl, 1 mM EDTA, 0.25% [w/v] sodium deoxycholate, 0.1% [v/v] Nonidet P-40, 1 mM NaF, 1 mM sodium pyrophosphate, 100 μM Na3 V04, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/ml aprotonin, and 0.7 μg/mL pepstatin). The suspension was centrifuged at 8,000g at 4°C for 10 minutes, and the protein content of the supernatant was determined by a Bradford assay, as previously described [13,17,18]. For RNA studies, total RNA was extracted using Trizol (Gibco BRL, Life Technologies, Inc., Grand Island, New York), according to manufacturer instructions. Tissue samples were homogenized in Trizol, incubated at room temperature and chloroform was added. After centrifugation at 13,000 × G for 20 minutes at 4°C the aqueous phase was collected and RNA was precipitated and washed by isopropyl alcohol and 75% ethanol, respectively. RNA concentration was determined by spectrophotometry, as previously described [13].

Vasoreactivity Studies

After equilibration at 0.3 g for 1 hour, a dose response curve for norepinephrine was obtained by cumulative addition of norepinephrine (10^{-9} to 10^{-4} M) in logarithmic increments. Strips were