Pharmacological Research 64 (2011) 431–437

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

Antioxidants to enhance fertility: Role of eNOS and potential benefits

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ARTICLE INFO

Article history:
Received 27 June 2011
Accepted 27 June 2011

Keywords:
Antioxidants
Infertility
Vascular dysfunction
Erectile dysfunction
Pre-eclampsia
Reproductive health
Nitric oxide

ABSTRACT

The use of antioxidants is now often used as a pharmacological adjunct to limit infertility. Indeed, the lay public rightly perceives oxidative stress and, thus, antioxidant treatment as important modulators of infertility. While the direct effects of antioxidant treatment on the quality of semen and oocytes are still under investigation, a significant body of evidence points to loss of vascular tone as a root-cause of erectile dysfunction and, possibly, alterations to female reproduction.

In this article, we will critically review the often neglected link between vascular dysfunction and infertility. A particular emphasis will be on the potential use of antioxidants to increase fertility and promote conception.

Contents

1. Introduction

Human reproduction is known to be a highly inefficient process where as much as 50% of conceptions fail, and ~20% of clinical pregnancies end in spontaneous abortion [1]. Both male and female reproductive dysfunction is thought contribute to this high rate of failure, but few defined etiologies have been identified. Despite the multifactorial nature of human infertility, there is a growing awareness that reactive oxygen and nitrogen species (ROS/RNS) and associated oxidative damage may be potent modulators of reproductive health [2,3]. Oxidative and nitrosative stress is associated with both risk factors for infertility (e.g. smoking, diabetes, hypertension, and aging), and directly in reproductive disorders as diverse as oocyte implantation, endometriosis, and pre-eclampsia in women, and erectile dysfunction, sperm damage and motility in men [2,4]. Because of this recognition, use of antioxidants and other redox modulatory compounds as nutraceutical/pharmacological treatments for many forms of infertility has soared as a result [3]. Numerous studies point to the benefits of dietary antioxidants to limit oxidative stress, thereby potentially maintaining the quality of semen and oocytes, and also lowering the risk for endometriosis and other female reproductive disorders. However, clinical trials

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doi:10.1016/j.phrs.2011.06.021

using antioxidants as therapies for many reproductive disorders have yielded negative or conflicting results as to their benefits on reproductive health [5–8]. While the direct effects of antioxidant treatment are still under investigation, it is interesting to note that many endpoints for both male and female infertility (e.g. erectile dysfunction in males, and preeclampsia, oocyte implantation, and endometriosis in women) actually involve vascular dysfunction, where antioxidants have provided significant benefits.

Herein we review the remarkable roles that vascular dysfunction plays as an underlying risk for many aspects of infertility as well as potential dietary antioxidant compounds that may improve vasomotor function, thereby limiting risk for infertility and promote conception.

2. Vascular dysfunction in conditions leading to infertility

The vascular endothelium that lines the lumen of vessels are critically important in maintaining vascular tone [9]. Endothelial cells are responsive to many stimuli that either promote vasorelaxation (e.g. shear stress, nitric oxide, prostacyclins, sphingosine 1-phosphate, acetylcholine, among others) or constriction (angiotensin II, endothelin-1, thromboxane) [10,11]. Thus, any condition, like oxidative stress, which impairs vasomotor function, could have profound adverse consequences on reproductive health.

Endothelial-derived vasorelaxation is gradually lost with age [12]. Consequently, endothelial dysfunction is a primary complication of age-dependent cardiovascular diseases, including hypertension, atherosclerosis, attendant coronary artery disease, the metabolic syndrome, and is increasingly recognized as a hallmark of congestive heart failure [13,14]. Impaired vasomotion is often observed in the absence of severe atherosclerotic lesions and endothelial dysfunction often occurs long before symptoms of coronary atherosclerosis become clinically apparent. It is often at this early stage of cardiovascular disease where clinical studies link endothelial impairment and infertility. For example, erectile dysfunction is a leading indicator of CVD [15], and erectile dysfunction patients display significantly impaired brachial artery flow-mediated dilation without additional cardiovascular risk factors [16,17].

Aside from erectile dysfunction, endothelial dysfunction also appears to be a significant factor in preeclampsia (pre-eclampsia), a hypertension syndrome occurring in 3–5% of pregnant women that results in reduced placental perfusion [18]. Pre-eclampsia is a primary event that significantly increases maternal and neonatal morbidity and mortality with no current therapy other than placental delivery. A number of studies now show a significantly reduced vasomotor function in pre-eclampsia patients. For example, Aardema et al. used Doppler analysis to show that the abnormal flow-mediated dilation of the uterine artery was strongly associated with pregnancy complications [19,20]. Brodszki and coworkers also showed that uterine artery flow was strongly impaired in pre-eclampsia with the lowest dilation evident in women with bilateral uterine artery notches [21]. Interestingly and in keeping with evidence for erectile dysfunction in men, no significant differences in carotid or aortic vessel wall stiffness are often evident in women with pre-eclampsia versus healthy controls. In summary, while pre-eclampsia is not a major cause of infertility, endothelial dysfunction contributes to this important disorder of pregnancy and should be adequately addressed.

From the available data, it is clear that infertility is multifactorial and should be individually addressed based on careful anamnesis [22]. However, infertility in both males and females appear to be linked by a profound loss in endothelial-dependent vascular function.

3. Vascular tone is principally regulated by endothelial-derived nitric oxide

As briefly mentioned above, the vascular endothelium governs vasoresponsiveness by physio-mechanical means, hormonal stimuli, and by synthesizing and releasing factors that act on the vascular smooth muscle layer [9]. The principal vasodilatory agent is nitric oxide (NO), which induces relaxation by stimulating cGMP production in vascular smooth muscle cells (VSMC). Often, loss of vasoresponsiveness has been attributed to insufficient endothelial-derived NO as exogenous NO donors (e.g. nitroprusside) restore adequate vasomotor function in CVD patients, the elderly, and in animal models [23]. Nitric oxide is synthesized by endothelial nitric oxide synthase (eNOS), one of a family of heme-containing, homodimeric enzymes that catalyze the 5 electron oxidation of the guanidinium moiety of L-arginine to NO and L-citrulline [24]. In endothelial cells, eNOS expression and activity is tightly governed both at the transcriptional and post-translational levels, by intracellular Ca^{+2} concentrations, and protein–protein interactions [25]. Additionally, its subcellular distribution also markedly affects overall enzyme activity. eNOS exists in at least two distinct subcellular locales in the endothelium: at caveolae of the plasma membrane and in the Golgi/perinuclear region of the cell [26]. Plasma membrane associated eNOS is considered to be more constitutively active and highly sensitive to agonist-induced intracellular Ca^{+2} fluxes. Additionally, eNOS at the plasma membrane is typically phosphorylated at Serine 1176 (*rattus* sequence) in an Akt-driven manner, which makes this caveolae-associated enzyme markedly more active in response to shear stress and other stimuli [27]. In contrast, Golgi-bound eNOS is less phosphorylated and relatively insensitive to Ca^{+2}-dependent activation; the latter may be due to inaccessibility of calmodulin to eNOS in this cellular compartment.

Besides subcellular locale, interactions of eNOS with effector proteins play a key regulatory role. eNOS in association with caveolin-1, a major protein component of endothelial caveolae, significantly inhibits eNOS activity as it prevents calmodulin binding [26,28]. Conversely, active eNOS at the plasma membrane can be co-immunoprecipitated with Hsp90 and kinases, especially Akt. Together, these regulatory mechanisms tightly govern eNOS-derived NO availability.

4. Protein effectors and the phosphorylation state of eNOS strongly affect its activity

Recent papers by Smith et al. [29,30] revealed that post-translational dysregulation of eNOS may play a remarkable role in endothelial dysfunction, especially in the elderly. These authors showed that aged rats displayed significant impairment in endothelial-derived vasomotor activity, but total eNOS content did not change with age. However, aging caused eNOS to disassociate from its activating proteins (Hsp90 and Akt) while increasing the levels of eNOS associating with caveolin-1, a protein known to inhibit eNOS activity. Along with this altered subcellular redistribution, these authors showed that the phosphorylation state of eNOS was significantly and persistently altered in aging rat aortic endothelia, which is consistent with its inactivation [29]. Loss of eNOS phosphorylation status was found to stem from chronically elevated sphingomyelinases (SMases) and resultant ceramide-driven protein phosphatase 2A (PP2A) activity, which specifically dephosphorylated S1176. Thus, age-related (and, likely, disease-related) changes in eNOS phosphorylation and/or its cellular association with other protein effectors may significantly alter eNOS activity and vascular function.