Roles of the Androgen–Androgen Receptor System in Vascular Angiogenesis

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Although many clinical studies have shown that a low testosterone level is associated with cardiovascular diseases, the role of androgens in cardiovascular physiology and pathophysiology remains controversial. Androgens exert various actions in their target organs, and the androgen receptor (AR) is widely distributed in several tissues, including endothelial cells, smooth muscle cells, and fibroblasts, in the vascular system. Biological activities of androgens are predominantly mediated through the AR by the transcriptional control of target genes and interaction with multiple signaling pathways. To clarify the molecular mechanisms of androgens in cardiovascular disease, we examined a pathological model using AR knockout mice and showed that the androgen–AR system has protective effects on cardiovascular remodeling against cardiovascular stress. In this review, we focus on the role of the androgen–AR system in angiogenesis after ischemic stress.


Key words: Androgen, Androgen receptor, Angiogenesis, Cardiovascular disease, Vascular endothelial growth factor

Introduction

The incidence of cardiovascular diseases (CVDs) is higher in men than in age-matched premenopausal women¹. Male sex is one of the major cardiovascular risk factors along with other traditional determinants such as hypertension, hyperlipidemia, diabetes mellitus, and smoking²,³. Previous studies have shown that testosterone replacement tends to increase cardiovascular risk among men of all ages⁴,⁶. However, serum testosterone levels exhibit gradual declines as men age⁷,⁸. Aging-related decline in serum androgen levels, referred to as “andropause” or “late-onset hypogonadism”⁹, causes a wide range of comorbid conditions such as a decrease in muscle mass and strength¹⁰, an increase in abdominal fat¹¹ (particularly visceral fat with insulin resistance¹²), atherogenic lipid profile¹³, type 2 diabetes mellitus¹⁴, and increased inflammatory biomarkers¹⁵. Recent epidemiological studies have revealed that lower testosterone levels in men are associated with higher mortality rates mainly due to CVDs¹⁶-²¹. Furthermore, recent evidence indicates that testosterone may be beneficial in the management of lifestyle-related disorders (e.g., obesity, metabolic syndrome and type 2 diabetes mellitus²²,²³) that are well known to be associated with an increased incidence of CVDs. Thus, a better understanding of the clinical and molecular links between androgens and CVDs is required.

Although the molecular mechanisms of androgen in the heart and blood vessels remain unclear, recent basic studies have provided evidences of the protective effects of androgens against CVDs. Analysis of the androgen receptor (AR) in vivo has revealed new aspects of the functional activities of androgens²⁴. We examined AR knockout mice and demonstrated that the androgen–AR system plays important roles in cardiovascular protection against cardiovascular stress, including angiotensin II and doxorubicin excess²⁵-²⁷.
In this review, we focus on the roles of the androgen–AR system in vascular angiogenesis after ischemic stress.

Androgen Actions are Mediated by AR Activation

Androgens are hormones secreted by the testis and adrenal gland that are critical for the development and maintenance of various organs as well as the male reproductive system. The major androgen in males is testosterone, and it can be converted to a more potent dihydrotestosterone (DHT) by the enzyme 5α-reductase. The actions of androgens are initiated through the AR, a member of the nuclear receptor superfamily. The AR is ubiquitously expressed in cells, including cells in the vasculature. Androgens bind to AR, and the liganded AR moves into the nucleus to bind to the AR-responsive elements in the target gene promoters; thus, it regulates the target gene expression at the transcriptional level. To induce the rapid activation of kinase-signaling cascades and up-regulation of cytosolic calcium concentration, androgens also exert their functions without transcriptional control through ARs in the cell membranes and cytoplasm. In fact, the acute vasodilatory action of testosterone is thought to be non-genomic that is independent of AR nuclear translocation and does not require protein synthesis.

Although the biological actions of testosterone are predominantly mediated by the AR, some may be mediated by the estrogen receptor (ER) activation after the conversion of testosterone to estradiol by aromatase. Animal models using castration or pharmacological interventions (except for non-aromatizable DHT) include the response of ER and AR dependent promoters; thus, it regulates the target gene expression at the transcriptional level. To induce the rapid activation of kinase-signaling cascades and up-regulation of cytosolic calcium concentration, androgens also exert their functions without transcriptional control through ARs in the cell membranes and cytoplasm. In fact, the acute vasodilatory action of testosterone is thought to be non-genomic that is independent of AR nuclear translocation and does not require protein synthesis.

Androgens are Associated with Arterial Diseases in Humans

A negative correlation between testosterone and blood pressure has been demonstrated in elderly men. Some studies have reported that the serum testosterone level is an independent negative predictor for the development of arterial stiffness and testosterone replacement therapy has been shown to improve arterial stiffness in hypogonadal men. These findings suggest that physiological androgen levels are associated with vascular relaxation and reactivity of blood vessels. Furthermore, observational studies revealed an inverse relationship between endogenous testosterone and coronary artery disease. Therapeutic use of testosterone in men with coronary artery disease improved cardiac ischemia. In elderly men, low testosterone levels have been shown to be associated with the development of peripheral artery disease (PAD). However, interventional studies on the role of androgen therapy in PAD remain a matter of debate. In addition, low levels of androgens in men have been shown to be important risk factors for the development of the intima-media thickening in the common carotid artery even after adjusting for age, sex, and other cardiovascular risk factors. Several human studies have also shown that the severity of aortic calcification negatively correlates with the testosterone serum level. Moreover, physiological concentrations of testosterone have been shown to maintain homeostasis of the hemostatic system through the enhancement of anticoagulant activity and antithrombotic effects. However, the protective effect of androgens on CVDs has remained controversial, and critical evaluation by further prospective studies is required.

Androgen–AR System Regulates Vascular Remodeling

Androgen actions on vascular function and inflammation may include the modulation of gene transcription via the AR and AR-mediated activation of a rapid intracellular signaling pathway. Vascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are pivotal cellular components of the vascular structure that play important roles in vascular health and disease. These cells express ARs and are targets for androgen action. Regarding ECs, it has been reported that androgens rapidly induce nitric oxide (NO) production in human endothelial cells via the phosphorylation/activation of endothelial nitric oxide synthase (eNOS) and that the activation of eNOS is inhibited by an antiandrogen drug or AR knockdown. Yu et al. demonstrated that testosterone induces the rapid assembly of a membrane-signaling complex among AR, c-Src, and caveolin-1, which facilitates activation of the c-Src/phosphoinositide-3-kinase (PI3K)/serine/threonine protein kinase (Akt) cascade, with the consequent activation of eNOS in vascular ECs. In addition, we have reported that AR deficiency impairs eNOS expression and phosphorylation in the murine aorta after angiotensin II loading. Cai et al. revealed that AR activation leads to proliferation of ECs, resulting in effective repair of ECs.
injury and prevention of EC dysfunction. In contrast, androgens have been shown to reduce proinflammatory cytokine production and adhesion molecular expression in several atherorelevant cells including monocytes, macrophages, ECs, VSMCs, and foam cells. Androgens are thought to modulate phenotypic changes of VSMCs through the upregulation of proliferation and interactions with intracellular signaling pathways induced by proinflammatory cytokines.

Several studies have revealed that androgens enhance tumor necrosis factor α and vascular cell adhesion molecule-1 expressions through interaction between the AR and nuclear factor-kappa B signaling pathway. Androgens also inhibit the production of interleukin 6, interleukin 1 beta, and expression of the intracellular adhesion molecule-1 and monocyte chemoattractant protein-1 in various cell types. We previously demonstrated that the AR provides protective effects against angiotensin II-induced vascular remodeling by regulating oxidative stress, c-Jun N-terminal kinase signaling, and transforming growth factor β phosphorylated-Smad pathway using AR knockout mice. Although a role of AR in the modulation of vascular cells is evident, the molecular mechanisms by which AR regulates vascular homeostasis and disease processes are not fully understood.

**Androgen–AR System Promotes Angiogenesis in Response to Ischemic Stress**

Angiogenesis is the formation of new blood vessels from the pre-existing endothelium, and it is physiologically and pathologically crucial for wound healing and organ regeneration in multiple disease processes. In certain situations, such as wound healing and ischemia, hypoxic tissues secrete various angiogenic proteins, including vascular endothelial growth factors (VEGFs) and fibroblast growth factors, to induce angiogenesis, thereby expanding the capillary network and increasing nutrient and oxygen supplies. VEGFs, major contributors to angiogenesis, induce EC differentiation, proliferation, migration, and survival, while promoting vascular permeability, and increase the number of capillaries in a given network.

Some studies have shown that androgens have a positive effect on angiogenesis in the repair of cardiovascular damage. Sieveking et al. demonstrated both in vitro and in vivo that androgens elicit an AR-mediated angiogenic effect in males but not in females after ischemia. They reported that castration led to a reduction in ischemia-induced angiogenesis in a male animal model. In addition, DHT, through activation of the AR, promoted in vitro angiogenic processes such as EC migration, proliferation, and tubulogenesis via VEGF-related mechanisms in ECs derived from male donors. In contrast, we demonstrated that both male and female AR knockout mice showed impaired revascularization after ischemia despite a robust increase of VEGF expression. Moreover, endothelial cells from AR knockout mice had attenuated angiogenic potency. The different gender-related findings in the studies of Sieveking et al. and our work may reflect the differences in animal models. Sieveking et al. used a castration model with wild-type mice, whereas we used AR knockout mice. Although we showed that AR deficiency impaired ischemic-induced angiogenesis also occurs in females, pathophysiological roles of the AR in females are largely unknown. In female AR knockout mice, serum concentrations of androgens and estrogens were the same as female wild-type mice in our experiment. Thus, we speculate that unliganded AR also plays a role in angiogenesis after ischemia regardless of sex. It has been reported that unliganded nuclear receptors, including thyroid hormone receptor, ER, and vitamin D receptor, have considerable biological actions. Further experiments are required to clarify this issue related to the physiological and pathophysiological roles of AR, independent of the estrogen effect in females.

**AR Plays a Pivotal Role in VEGF-Stimulated Akt and eNOS Signaling Pathways**

VEGFs mediate their effects on ECs by activating cell VEGF receptor (VEGFR) tyrosine kinases. In general, VEGF-A activation of VEGFR2 (also known as kinase insert domain protein receptor (KDR) in humans) is the main regulator of blood EC function. The PI3K/Akt pathway is activated by VEGF and is capable of modulating cell survival, migration, tube formation, and NO release. Akt activates eNOS and enhances NO production, and the release of NO from vascular endothelial cells is essential for promoting angiogenesis and collateral vessel remodeling in response to ischemia. Much research has focused on the NO-mediated vasoreactive mechanisms of testosterone in penile tissue with regard to sexual function and erectile dysfunction. Androgens increase the expression of eNOS and enhance NO production through genomic and nongenomic effects of the AR in human umbilical vein endothelial cells (HUVECs), effects that were significantly reduced by AR blockade. We also found that VEGF-stimulated Akt and that eNOS phosphor-
vation was blunted by AR deficiency in vivo and in vitro\(^60\). Because the ischemic muscle of AR-deficient mice showed the attenuation of Akt-eNOS phosphorylation and the reduced activation of Akt and eNOS attenuated angiogenesis in the animal model, the AR is required for promoting angiogenesis partly through activation of the PI3K/Akt-eNOS signaling pathway in response to ischemic stress (Fig. 1). This result is consistent with the results of our previous study showing that AR deficiency causes a reduction in eNOS expression and phosphorylation, leading to the attenuation of NO bioavailability and acceleration of vascular remodeling under the condition of angiotensin II excess\(^67\). Recently, Lecce et al. demonstrated that aging is associated with the impairment of DHT-mediated regulation of VEGF-dependent angiogenesis, a finding characterized by androgen insensitivity with age-related defective nuclear translocation of the AR with androgen exposure\(^84\). In addition, other studies have demonstrated that testosterone ameliorates endothelial senescence, through eNOS/sirtuin 1-dependent mechanisms in vitro\(^85\), and sirtuin 1 in ECs plays important roles in angiogenesis\(^86\). There is a possibility that sirtuin 1 participates in impaired angiogenesis via the attenuation of Akt-eNOS activation after ischemia in AR knockout mice.

The activation of VEGF–VEGF receptor signaling is critical for the angiogenic response to ischemia\(^87\). Sieveking et al. reported that DHT induced dose-dependent increases in the mRNA expression of KDR, a major VEGF receptor, in male HUVECs\(^75\). We also demonstrated that AR activation is required for the compensatory up-regulation of KDR gene expression in response to ischemia, at least in male mice\(^36\). Previous studies revealed that a ternary complex formed among AR, PI3Kp85, and Src is essential for the androgen-stimulated activation of PI3K/Akt and mitogen-activated protein kinase\(^88, 89\) and that KDR is activated by the recruitment and activation of Src and PI3K\(^90\). Recently, we demonstrated that there is a novel cross-talk mechanism between androgen/AR signaling and VEGF/KDR signaling pathways through the recruitment and activation of signaling molecules including PI3Kp85 and Src in HUVECs (as illustrated in Fig. 1). Although further studies are required to reveal the detailed interaction mechanism between AR and KDR, there is a possibility that AR and KDR share these same signaling molecules and that AR activation and association with KDR play an important role in full activation of the KDR signaling pathway in response to ischemia\(^36\).

![Fig. 1. Schematic representation of associations between AR and VEGF receptor signaling pathways](image)

**VEGF rapidly induces KDR activation followed by the recruitment and activation of Src and PI3K.** On the other hand, AR activates the Akt/eNOS cascade depending on the association with PI3K and Src. Thus, the association between AR and KDR promotes cell survival and angiogenesis via the recruitment of Src and PI3K.

**AR does not have any Influence on Bone Marrow-Derived Endothelial Progenitor Cells**

Bone marrow-derived endothelial progenitor cells (EPCs), an integrated component of the cardiovascular system, are mobilized to peripheral circulation in response to multiple stimuli, and they participate in revascularization in ischemic tissues\(^39\). EPCs are postulated to contribute to the repair of damaged endothelium by incorporation into the vessel wall, secretion of paracrine hormones, and stimulation of angiogenesis\(^93\). Thus, depletion and/or dysfunction of EPCs are pathogenic events that contribute to the inability to maintain an intact endothelium and to promote angiogenesis\(^93\). Human EPCs express ARs, and androgens increase EPC mobilization from bone
marrow in testosterone replacement therapy\textsuperscript{94, 95}. Results of in vitro studies revealed that testosterone increased EPC proliferation, colony formation, and migration through the activation of AR-mediated pathways\textsuperscript{95}. Sieving et al. also reported that androgens promote the mobilization of bone marrow progenitor cells that can theoretically promote revascularization via the paracrine secretion of angiogenic factors in an animal model\textsuperscript{75}. Moreover, the PI3K/Akt pathway was shown to play an important role in androgen-modulated EPC proliferation and adhesion\textsuperscript{96}. However, our bone marrow transplantation study using AR knockout mice demonstrated that AR-deficient bone marrow progenitor cells have no impact on angiogenesis in response to ischemia\textsuperscript{96}.

Conclusions and Clinical Perspective

Not only menopause but also andropause is a crucial risk factor for the development of CVDs. Here we showed that AR activation exerts protective roles in vascular remodeling and angiogenesis against critical vascular stress. Although favorable biological effects of androgens on various tissues, including the bones, skeletal muscles, fat, and central nervous system, have been studied in detail, the practical use of androgens has not become widespread because of their undesirable side effects, including risk of prostate cancer in men and hirsutism in women\textsuperscript{97}. Several recent reports have indicated that testosterone replacement therapy may produce cardiovascular risks\textsuperscript{98}, whereas others report no risk\textsuperscript{99, 100}. Some evidence suggests that testosterone levels titrated to within the mid- to upper-normal range have at least neutral or beneficial effects of testosterone on a series of cardiovascular risk factors\textsuperscript{101, 102}, therefore, there may possibly be a cardioprotective action. Further large randomized placebo-controlled trials are required to elucidate its long-term clinical relevance to cardiovascular health in men\textsuperscript{103}.

Selective androgen receptor modulators (SARMs) are agents with desirable androgenic effects in certain tissues, e.g., muscle and bone; notably they produce these effects without impacting other organs, like the prostate or skin, thereby limiting the adverse effects typically associated with androgens (prostate growth or androgenization)\textsuperscript{104}. More recently, SARMs have been under clinical development, and trials are underway in patients with sarcopenia and cachexia\textsuperscript{105}. On the basis of the results of previous studies and our recent studies, we believe that appropriately targeted regulation of AR function in the cardiovascular system is unable to develop novel therapeutic approaches for the treatment of CVDs.

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Abbreviations

Akt, serine/threonine protein kinase; AR, androgen receptor; CVD, cardiovascular disease; DHT, dihydrotestosterone; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; ER, estrogen receptor; HUVEC, human umbilical vein endothelial cell; KDR, kinase insert domain protein receptor; NO, nitric oxide; PAD, peripheral arterial disease; PI3K, phosphoinositide-3-kinase; SARM, selective androgen receptor modulators; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VSMC, vascular smooth muscle cell

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