REVIEW

Effects of androgens on cardiovascular remodeling

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Abstract

Androgens, the male sex hormones, exert various biological effects on many target organs through the transcriptional effects of the nuclear androgen receptor (AR). ARs are expressed not only in classical target organs, such as the brain, genital organs, bone, and skeletal muscles, but also in the cardiovascular system. Because the female sex hormones estrogens are well-known to protect against cardiovascular disease, sex has been considered to have a significant clinical impact on

cardiovascular mortality. However, the influence of androgens on the cardiovascular system has not been fully elucidated. To clarify this issue, we analyzed the effects of administration of angiotensin II and doxorubicin, an anticancer agent, in a loading model in male wild-type and AR-deficient mice. In this review, we focus on the actions of androgens as potential targets for the prevention of cardiovascular diseases in males.

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Introduction

Cardiovascular disease remains a major cause of death in both women and men worldwide and appears to increase morbidity and mortality in industrial countries. The various risk factors for development of cardiovascular disease include aging, hypertension, dyslipidemia, diabetes, obesity, and smoking. In addition to these cardiovascular risk factors, there are several sex differences in cardiovascular disease. The incidence of cardiovascular disease is approximately twofold higher in males than in females (Kalin & Zumoff 1990). This sex discrepancy in cardiovascular disease rates has been thought to be associated with sex hormone-mediated actions. Estrogens, which are female sex hormones, are generally considered to exert favorable effects against cardiovascular diseases. Many studies have revealed preventive and favorable effects of estrogen on cardiac hypertrophy (Malhotra *et al.* 1990, Weinberg *et al.* 1999, van Eickels *et al.* 2001, Cavašin *et al.* 2003) and vascular remodeling (Zhang *et al.* 2000).

In contrast, male sex is generally believed to be one of the major cardiovascular risk factors, along with other traditional determinants such as hypertension, hyperlipidemia, diabetes, and smoking (Criqui 1986). Therefore, the male sex hormones, androgens, are thought to be detrimental to cardiovascular organs. In fact, androgen replacement is associated with cardiovascular-related adverse events in aged men who have limitations in mobility (Basaria *et al.* 2010).

In addition, previous studies have shown that testosterone replacement tends to increase cardiovascular risk among men of all ages (Calof *et al.* 2005, Haddad *et al.* 2007, Fernandez-Balsells *et al.* 2010). On the other hand, recent epidemiological studies have revealed that lower testosterone levels in men are associated with higher mortality rates, due largely to cardiovascular disease (Khaw *et al.* 2007, Laughlin *et al.* 2008, Tivesten *et al.* 2009, Yeap *et al.* 2009, Akishita *et al.* 2010, Corona *et al.* 2010, Malkin *et al.* 2010, Araujo *et al.* 2011; Fig. 1). Moreover, patients undergoing androgen-deprivation therapy for prostate cancer are at increased risk of coronary heart disease and heart failure (Martin-Merino *et al.* 2011). It has been reported that low testosterone is associated with metabolic syndrome or diabetes in men (Stanworth & Jones 2009, Grossmann 2011). Prospective studies have also revealed that men with higher testosterone level have a low risk of type 2 diabetes (Ding *et al.* 2006).

Although the mechanisms of the heart- and blood vessel-protective activities of androgens remain unclear, recent studies have shown preventive effects of androgens against cardiovascular disease. In addition, analysis of androgen receptor (AR)-knockout (KO) mice (Sato *et al.* 2003) has revealed new aspects of the functional activities of androgens in various tissues, including adipocytes (Sato *et al.* 2003, Fan *et al.* 2005), brain (Sato *et al.* 2004), bone (Kawano *et al.* 2003), and cardiovascular organs (Ikeda *et al.* 2005, 2009, 2010). Thus, the androgen-AR system plays an important

Figure 1 Relationship between serum testosterone level and cardiovascular mortality in men. Reduced levels of endogenous testosterone worsen the mortality of cardiovascular disease in men, indicating that testosterone is a predictive factor of cardiovascular disease. (Modified from *Khaw et al. (2007)*).

role in homeostasis of various organs in males, although the favorable effects of androgens remain controversial.

In this review, we give an overview of the pathophysiological roles of androgen activity in the cardiovascular system and focus on the cardiovascular stress-induced phenotypes observed in male ARKO mice in order to elucidate the effect of AR against cardiovascular diseases.

Male sex hormones, androgens

Androgens in the human body comprise testosterone, dihydrotestosterone (DHT), androstenedione, and dehydroepiandrosterone (DHEA) and its sulfate DHEAS. Most of the testosterone is secreted by the testis. Approximately 5% of the serum testosterone produced in men undergoes 5 α -reduction to form a more potent androgen, DHT. DHT has threefold greater affinity than testosterone and 15- to 30-fold greater affinity than adrenal androgens for ARs. DHEA and DHEAS, the most abundant adrenal steroids in humans, are precursors for intracellular production of androgens and estrogens in nonreproductive tissues. Most of the DHEA in blood exists as DHEAS, with $\sim$ 300-fold more DHEAS than free DHEA. Therefore, DHEAS is the most physiologically active of this class of adrenal steroids in humans. Most testosterone exists as forms bound to plasma proteins, including 40–50% bound to albumin and 50–60% strongly bound to sex hormone-binding globulin (SHBG), with 1–2% being free (*Dunn et al. 1981*). Both free testosterone and albumin-bound testosterone are fractions available for biological action, so-called bioavailable testosterone. On the other hand, SHBG-bound testosterone is not a readily bioactive form (*Cefalu et al. 1986*). DHT also strongly binds to SHBG, and only 0.8% is a free

fraction. Other androgens including DHEA and DHEAS are bound to albumin (*Plager 1965*).

The levels of all androgens increase at puberty and peak during adolescence, then gradually decrease with age (*Giusti et al. 1975*).

Mechanisms of androgen activity via AR activation

The actions of androgens are initiated by the binding of androgens to the AR. AR, a 110-kDa ligand-inducible nuclear receptor, is a member of the nuclear receptor superfamily. The effects of androgens are generally mediated by AR activation (*Chang et al. 1988a,b, Orlic et al. 2001*). The biological actions of androgens via transcriptional regulation of target genes by the AR are referred to as 'genomic action' (*Orlic et al. 2001*). Initially, androgen enters the cells and binds to AR; the ligand-bound AR then dimerizes and translocates into the nucleus, and binds to specific androgen response element sites located within the promoter regions of the target genes to modulate their transcriptional activities. Androgens also exert effects through extranuclear–nontranscriptional action, known as nongenomic action (*Losel et al. 2003, Simoncini & Genazzani 2003*). Nongenomic androgen effects are distinct from genomic action in that they are exerted for a shorter time. Nongenomic androgen activity involves rapid induction of second messenger signal transduction cascades, including upregulation of cytosolic calcium concentration and activation of protein kinase A, protein kinase C (PKC), and MAP kinases (*Kousteni et al. 2002*). The nongenomic action of androgen might exert, in part, through stimulating protein kinase A and cAMP via binding to G-protein coupled membrane receptor for the SHBG–testosterone complex (*Heinlein & Chang 2002; Fig. 2*).

AR expression in cardiovascular tissues

Androgens exert biological effects on many target organs (*Mooradian et al. 1987, Wilson 1999*). While AR expression is highest in male sex organs (*Wilson & French 1976*), the AR is known to be widely if weakly expressed in other tissues, including skeletal muscle (*Snochowski et al. 1980*), bone (*Riggs et al. 2002*), and the brain (*McGill et al. 1980*). The AR is also found in cardiac myocytes (*McGill et al. 1980, Marsh et al. 1998*), endothelial cells, vascular smooth muscle cells, and fibroblasts (*Horwitz & Horwitz 1982, Lin et al. 1982*). Therefore, androgen–AR effects are also thought to be exerted on the heart and vasculature.

Androgen effects on cardiac remodeling

The AR gene is expressed in mammalian cardiomyocytes (*McGill et al. 1980, Marsh et al. 1998*), indicating that androgens might have a function in the heart. The estrogen

Figure 2 Biological actions of androgens via ARs. Androgen binds to the AR. The liganded AR forms homodimers and moves it into the nucleus to bind to specific DNA elements referred to androgen-responsive elements in target gene promoters and regulates the target gene expression at the transcriptional level. This action of the AR is called genomic action. Androgen-AR also exerts its function without transcription by interaction with other signaling pathways. This action is called nongenomic action.

receptor gene also exists in cardiomyocytes (Grohe *et al.* 1997, 1998), and many studies have shown preventive effects of estrogen on cardiac hypertrophy (Malhotra *et al.* 1990, Weinberg *et al.* 1999, Cavašin *et al.* 2003). However, the physiological actions of androgens in the heart have remained unclear compared with those of estrogen. Marsh *et al.* (1998) have shown that androgen exerts a hypertrophic effect on cardiac myocytes via a direct AR-mediated pathway, and other studies have also shown that androgens induce cardiac hypertrophy (Malhotra *et al.* 1990, Weinberg *et al.* 1999, Cavašin *et al.* 2003), while castration (Morano *et al.* 1990, Cavašin *et al.* 2003, Li *et al.* 2004) and flutamide (Baltatu *et al.* 2002), an AR antagonist, remarkably reduce cardiac hypertrophy. In addition, androgens modulate male cardiac performance by regulating the functional expression of L-type calcium channels in cardiac myocytes (Golden *et al.* 2003). Li *et al.* (2004) showed that castration mitigated not only cardiac hypertrophy but also cardiac fibrosis in male guanylyl cyclase-A-deficient mice.

In the clinical setting, male patients with chronic heart failure exhibit declines in serum androgen levels (Anker *et al.* 1997, Kiilavuori *et al.* 1999, Moriyama *et al.* 2000, Kontoleon *et al.* 2003), and deficiencies in circulating androgens, including testosterone, in men with chronic heart failure have been shown to be independent predictors of poor outcome (Jankowska *et al.* 2010). On the other hand, administration of testosterone with moderate heart failure has been shown to reduce left ventricular mass index, indicating the amelioration of cardiac hypertrophy without

improving cardiac function. The effects of physiological androgen levels on cardiac remodeling and function thus remain controversial, and the molecular mechanisms of the underlying effects of androgens on the heart are still a matter of debate.

Approach for study of androgen activity: overview and aberrant cardiac remodeling in ARKO mice

The effects of androgens on the cardiovascular system are usually studied in animal models using castration or pharmacological interventions. While gonadectomy is indeed an easy way to eliminate androgen activity *in vivo*, the AR itself is still present in the castrated animal model. Flutamide, an AR antagonist, has been used for treatment of prostate cancer and also to clarify the influence of androgen activity blockade in numerous experimental studies. However, flutamide activates estrogen receptors, complicating the understanding of AR action. To elucidate the physiological function of the androgen-AR system in nonclassical target organs *in vivo*, we used the Cre-loxP system to generate ARKO mice (Sato *et al.* 2003). Male ARKO mice exhibit extremely low serum levels of testosterone and DHT, while estrogen levels are similar between male ARKO and wild-type (WT) mice. Therefore, ARKO mice are a unique animal model with a normal estrogen-ER system. Studies using ARKO mice have in recent years provided several new insights into the androgen-AR system. Male ARKO mice

demonstrate late-onset obesity (Sato *et al.* 2003), with decreased energy expenditure and enhanced insulin sensitivity (Fan *et al.* 2005), disordered hypothalamic leptin signaling (Fan *et al.* 2008), high turnover osteopenia (Kawano *et al.* 2003), impaired brain masculinization (Sato *et al.* 2004), dysregulation of the pituitary glucocorticoid feedback system (Miyamoto *et al.* 2007), enhanced hair growth (Naito *et al.* 2008), and altered skeletal muscle strength (Chambon *et al.* 2010). ARKO mice are thus a useful tool for investigating androgen–AR activity in classical and nonclassical target organs and may have advantages over conventional models such as gonadectomized animals or administration of AR antagonists. Therefore, we used male ARKO mice to clarify the physiological role of the androgen–AR system in the heart. AR regulates physiological cardiac growth and

modulates cardiac adaptive hypertrophy and fibrosis during angiotensin II (Ang II)-induced cardiac remodeling (Fig. 3; Ikeda *et al.* 2005). Ang II-treated ARKO mice showed the reduced activation of extracellular signal-regulated kinases (ERKs) 1/2 and 5, hypertrophy-related signaling pathways, acceleration of the transforming growth factor- β 1 (TGF- β 1)–Smad pathway, and increased expression of genes related to fibrotic change in the heart. The AR also counteracts doxorubicin (Dox)-induced cardiotoxicity, in part via activation of the Akt pathway, which upregulates TFAM and reduces oxidative stress and thus protects cardiac myocytes against mitochondrial damage and apoptosis (Fig. 4; Ikeda *et al.* 2010). These findings might account for the effect of testosterone replacement in male patients with severe heart failure (Malkin *et al.* 2006).

Figure 3 The AR system participates in physiological cardiac growth and inhibits angiotensin II-induced cardiovascular remodeling. (Modified from Ikeda *et al.* (2005, 2009)).

Figure 4 ARKO mice are susceptible to Dox-induced cardiotoxicity. (Upper panels) Echocardiography shows greater reduction of cardiac systolic function in Dox-treated ARKO mice than in Dox-treated WT mice. (Middle panels) Electron microscopy shows larger numbers of cardiac vacuoles and abnormal mitochondrial formation in Dox-treated ARKO mice. (Lower panels) Dox-induced cardiac superoxide production is accelerated in Dox-treated ARKO mice relative to Dox-treated WT mice. (Modified from Ikeda *et al.* (2010)).

Effects of androgens on vascular remodeling

The effects of androgens on blood vessels are also controversial. As male sex is one of the major risk factors for the development of cardiovascular disease, it is hypothesized that androgens promote atherosclerosis. In fact, several *in vivo* and *in vitro* studies have suggested that androgens increase expression of proatherogenic factors (Adams *et al.* 1995, McCrohon *et al.* 1999, Ng *et al.* 2003, Nheu *et al.* 2011). In contrast, recent clinical studies have shown that low endogenous testosterone levels are associated with advanced atherosclerosis of the carotid artery in middle-aged males and that this association is independent of the traditional cardiovascular risk factors (Muller *et al.* 2004, Makinen *et al.* 2005). Low testosterone level is also associated with poor vasodilation of the brachial artery and is an independent risk factor for endothelial dysfunction in men (Akishita *et al.* 2007). Androgen replacement has been shown to prevent aortic atherosclerotic changes in castrated male rabbits fed a high cholesterol diet (Alexandersen *et al.* 1999), and arterial AR mRNA upregulation by testosterone has been shown to reduce neointimal plaque formation in male rabbits (Hanke *et al.* 2001). Endogenous testosterone also inhibits coronary neointimal formation after balloon injury through enhanced expression of PKC δ and p27 (kip1; Sharp *et al.* 2009). Qiu *et al.* (2010) showed that physiological levels of DHT attenuated the development of atherosclerosis by AR-mediated suppression of the formation of intimal foam cells by macrophages. Furthermore, we have reported that DHEAS level is inversely associated with carotid atherosclerosis, as measured by increased max-intima-media thickness (IMT) and mean-IMT, in males but not in females (Yoshida *et al.* 2010). Therefore, in order to elucidate the pathophysiological roles of AR activity in the cardiovascular

system, we studied male ARKO mice under vascular stress (Ikeda *et al.* 2009). Male ARKO mice exhibited exaggerated Ang II-induced medial thickening and perivascular fibrosis in the coronary artery and aorta. Ang II-induced oxidative stress was exacerbated and nitric oxide bioavailability decreased in male ARKO mice. Androgen activity thus influences Ang II-induced vascular remodeling by suppressing oxidative stress and preserving nitric oxide production (Ikeda *et al.* 2009). Androgen-AR system-promoted eNOS activation was mediated by both phosphatidylinositol 3-kinase (PI3K)/Akt signaling and the direct interaction of AR with p85 α (Ikeda *et al.* 2010, Koizumi *et al.* 2010). In addition, AR deletion in Apo-E-deficient mice accelerated the atherosclerotic changes; this effect was partially attenuated by androgen supplementation (Bourghardt *et al.* 2010). Interestingly, physiological testosterone supplementation inhibited cholesterol-enriched diet-induced fatty streak formation in Tfm mice, which have a deletion in the gene encoding the classical AR (Nettelship *et al.* 2007). These results suggested that androgen exerts an atheroprotective effect via AR-dependent and -independent signaling. The androgen-AR system thus protects vascular remodeling through multiple signaling pathways.

The effects of androgens on angiogenesis

While the effect of estrogens on angiogenesis has been studied extensively and characterized in detail (Losordo & Isner 2001), very little is known about the effect of androgens on angiogenesis. As proof that the androgen-AR system is involved in angiogenesis, Sieveking *et al.* (2010a) demonstrated both *in vitro* and *in vivo* that androgens elicit an AR-mediated angiogenic effect in males but not in females.

Figure 5 Schematic diagram of cardiovascular-protective effects of ligand-bound AR.

They concluded that androgens promote angiogenesis via vascular endothelial growth factor (VEGF)-related mechanisms (Cai *et al.* 2011) and also by stimulation of erythropoietin production (Sieveking *et al.* 2010a,b). Further studies are necessary to clarify the significance of androgens in angiogenesis.

Androgens and peripheral arterial disease

Peripheral arterial disease (PAD) not only decreases quality of life due to intermittent claudication, but also, more importantly, is a powerful predictor of future cardiovascular and cerebrovascular events such as myocardial infarction, stroke, and death (Smith *et al.* 1990, Criqui *et al.* 1992, Murabito *et al.* 2003). Previous clinical studies have shown that PAD is two- to three-fold more prevalent in men than in women (Dormandy & Rutherford 2000) and that low testosterone levels are associated with lower extremity PAD in elderly men (Tivesten *et al.* 2007). Conversely, it has been reported that sex hormones are not associated with the development of PAD in men or postmenopausal women (Price *et al.* 1997). Therefore, the pathophysiological roles of androgen activity in the development of PAD remain a matter of debate.

Androgens and hypertension

It has been believed that blood pressure is higher in males than in females after puberty (Burt *et al.* 1995) and that there are sex differences in the prevalence and complications of hypertension. Indeed, patients undergoing androgen-deprivation therapy due to prostate cancer showed decreased

central arterial compliance (Dockery *et al.* 2000), and several studies have shown that low serum testosterone level is associated with high blood pressure in elderly men (Fogari *et al.* 2005, Akishita *et al.* 2010) and that suppression of testosterone production increases arterial stiffness (Dockery *et al.* 2003). In addition, testosterone replacement therapy has been shown to improve arterial stiffness in elderly hypogonadal men (Yaron *et al.* 2009). These findings suggest that physiological androgen levels preserve appropriate blood pressure by modulating vascular stiffness.

In experimental animal models, castration reduced blood pressure in male rats with spontaneous (Martin *et al.* 2005, Ojeda *et al.* 2007) or fructose feeding-induced (Song *et al.* 2004) hypertension. Testosterone has also been shown to inhibit L-type calcium channels via an AR-independent pathway, indicating nongenomic action of androgen on vasodilatation (Scragg *et al.* 2004, 2007, Hall *et al.* 2006). However, we have found that there is no difference in blood pressure between male ARKO and age-matched WT mice at 6 months of age (Ikeda *et al.* 2005). As the results of these animal studies are inconsistent with the results of clinical human studies, further research on the mechanisms by which androgens affect blood pressure is required.

Conclusion

Although androgens, in contrast to estrogen, have been considered to have adverse effects on the cardiovascular system, recent studies have revealed favorable effects of androgens on cardiovascular remodeling. Based on these previous results, at least physiological levels of androgens are thought to be required for cardiovascular homeostasis in males. As a better understanding of the sex differences in

cardiovascular diseases might lead to novel therapeutic strategies, the pathophysiology of the involvement of sex hormones in the cardiovascular system must be determined in detail. Figure 5 shows a conceptual summary of the currently known pathophysiological activities of ligand-bound AR described in this review.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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