REVIEW

Testosterone as a protective factor against atherosclerosis – immunomodulation and influence upon plaque development and stability

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Abstract

Inflammation plays a central pathogenic role in the initiation and progression of coronary atheroma and its clinical consequences. Cytokines are the mediators of cellular inflammation and promote local inflammation in the arterial wall, which may lead to vascular smooth muscle apoptosis, degradation of the fibrin cap and plaque rupture. Platelet adhesion and thrombus formation then occur, resulting clinically in unstable angina or myocardial infarction. Recent studies have suggested that cytokines are pathogenic, contributing directly to the disease process. ‘Anti-cytokine’ therapy may, therefore, be of benefit in preventing or slowing the progression of cardiovascular disease. Both oestrogens and testosterone have been shown to have immune-modulating effects; testosterone in particular appears to suppress activation of pro-inflammatory cytokines. Men with low testosterone levels are at increased risk of coronary artery disease. An anti-inflammatory effect of normal physiological levels of sex hormones may, therefore, be important in atheroprotection. In this article, we discuss some of the mechanisms involved in atherosclerotic coronary artery disease and the putative link between testosterone deficiency and atheroma formation. We present the hypothesis that the immune-modulating properties of testosterone may be important in inhibiting atheroma formation and progression to acute coronary syndrome.

Cytokines in coronary artery disease

It has become clear in recent years that the clinical events of the acute coronary syndromes in patients with coronary artery disease (CAD) result from a change from quiescent atheroma to ruptured plaque in which inflammation within the vessel wall is intimately associated (Libby et al. 2002). Patients with CAD have elevated circulating levels of cytokines and C-reactive protein (CRP) (Ikonomidis et al. 1999), with higher levels present in those with unstable angina or acute myocardial infarction (Manten et al. 1998). The CRP level, a marker of cytokine activation, is not only a strong independent predictor of mortality and subsequent events in patients admitted to hospital with an acute infarct (Tommasi et al. 1999) but is also strongly associated with significant atheromatous disease in unselected ‘healthy’ populations (Ridker et al. 1997). It was previously thought that the degree of coronary artery narrowing was the main predictor of subsequent events, but angiographic studies have shown that most infarcts occur in vessels with stenoses of less than 70% (Falk et al. 1995). Thus, the clinical manifestations of coronary atheroma are not directly related to the local burden of atheroma but to the amount of plaque inflammation. Moreover, statins reduce plasma lipid levels and coronary events and alter plaque constituents but have only a minimal effect on the degree of stenosis (Jukema et al. 1995). It would appear, therefore, that it is the quality and not the quantity of plaque which is most important.

The initial process of atheroma formation involves accumulation of cholesterol in the arterial wall and expression of adhesion molecules and chemokines by endothelial cells, in particular monocyte chemoattractant protein-1 (MCP-1) (Reckless et al. 1999). As a consequence, monocytes and macrophages infiltrate the vessel wall and produce inflammatory cytokines including tumour
The stability of the fibrous cap may be threatened when the rate of extracellular matrix breakdown exceeds the rate of synthesis. This may occur as a consequence of excess cytokine-induced production of MMPs or due to increased rate of vascular smooth muscle cell apoptosis. Rupture of the fibrous cap most often occurs where the layer of vascular smooth muscle cells is thinnest, highlighting their importance (Davies & Thomas 1985). The relative rates of vascular smooth muscle cell proliferation and apoptosis are critical in governing plaque stability (Fig. 2). Vascular smooth muscle cells taken from plaques show higher rates of apoptosis (Geng & Libby 1995), have senescent morphology (Orekhov et al. 1984) and are more sensitive to apoptotic stimuli than smooth muscle cells from normal vascular tissue (Bennett et al. 1995). The common areas of plaque rupture - the shoulders of the plaque - have the highest density of apoptotic vascular smooth muscle cells. Once again, inflammatory cells and their cytokines are the key. Macrophages can directly induce vascular smooth muscle cell apoptosis (Boyle et al. 2001), as can TNF, IL-1β and interferon-γ (Geng et al. 1996).

**Testosterone and coronary artery disease**

Men are more than twice as likely as women to die from coronary heart disease, and this ratio is consistent in all populations and is not related to differences in risk factors (British Heart Foundation Statistics Database 1998). Sex hormones decline with age in both sexes but the relationship of sex hormones to cardiovascular risk is complex. Pre-menopausal women have a lower incidence of CAD, but this rises after menopause so that the risk rapidly approaches that of males. One explanation for this phenomenon is that sex hormones influence the development and progression of coronary artery disease. In males the increasing incidence of CAD with age is associated with a decline in testosterone levels. A similar, more pronounced epidemiological pattern occurs in women after the menopause, so that there is a catch-up time of around 10 years. The presumed protective effects of oestrogens in women have been widely investigated with a series of large observational studies tending to support this notion (Gordon et al. 1978, Stampfer et al. 1991). However, randomised control trials designed to assess the effect of female hormone replacement on cardiovascular events have reported either no benefit (Hulley et al. 1998) or harm (Rossouw et al. 2002). The explanation for these seemingly opposing results is unclear. The positive observational data have been attributed to ‘compliance bias’ whereby women on hormone replacement therapy (HRT) tend to be healthier, from a higher socio-economic class and exhibit fewer cardiovascular risk factors (Petitti 1998). The negative trial data of HRT in women with established vascular disease (HERS) (Hulley et al. 1998)

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**Table 1** Actions of tumour necrosis factor

<table>
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<tr>
<th>In vitro</th>
<th>Impaired endothelial nitric oxide synthase production</th>
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<tr>
<td></td>
<td>Increased inducible nitric oxide synthase production</td>
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<tr>
<td></td>
<td>Impaired vasodilation</td>
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<td></td>
<td>Reduced myocyte contractility</td>
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<td></td>
<td>Increased metalloproteinase activity</td>
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<td></td>
<td>Reduced TIMP activity</td>
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<tr>
<td></td>
<td>Promotes apoptosis</td>
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<td></td>
<td>Induces expression of IL-1β, IL-6, MCP-1</td>
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<table>
<thead>
<tr>
<th>In vivo</th>
<th>Impaired left ventricular function</th>
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<tr>
<td></td>
<td>Left ventricular dilation</td>
</tr>
<tr>
<td></td>
<td>Heart failure</td>
</tr>
<tr>
<td></td>
<td>Impaired peripheral vasodilation</td>
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<td></td>
<td>Protein breakdown</td>
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<td></td>
<td>Impaired collagen synthesis</td>
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TIMP, tissue inhibitor of matrix metalloproteinase; IL, interleukin; MCP, monocyte chemoattractant protein.

The stability of the fibrous cap may be threatened when the rate of extracellular matrix breakdown exceeds the rate of synthesis. This may occur as a consequence of excess cytokine-induced production of MMPs or due to increased rate of vascular smooth muscle cell apoptosis. Rupture of the fibrous cap most often occurs where the layer of vascular smooth muscle cells is thinnest, highlighting their importance (Davies & Thomas 1985). The relative rates of vascular smooth muscle cell proliferation and apoptosis are critical in governing plaque stability (Fig. 2). Vascular smooth muscle cells taken from plaques show higher rates of apoptosis (Geng & Libby 1995), have senescent morphology (Orekhov et al. 1984) and are more sensitive to apoptotic stimuli than smooth muscle cells from normal vascular tissue (Bennett et al. 1995). The common areas of plaque rupture - the shoulders of the plaque - have the highest density of apoptotic vascular smooth muscle cells. Once again, inflammatory cells and their cytokines are the key. Macrophages can directly induce vascular smooth muscle cell apoptosis (Boyle et al. 2001), as can TNF, IL-1β and interferon-γ (Geng et al. 1996).
has been attributed to the higher use of statins in the control group (Pan & Boal 1999) and to late randomisation of subjects (with consequent failure to prove a late trend to cardio-protection) (Ong et al. 1999). The Women’s Health Initiative study (Rossouw et al. 2002) in unselected ‘healthy’ women also found no benefit and indeed small absolute increases in the risk of vascular disease and cancer. This trial has also been criticised for the high age of the study population, with 50% over 65 years old and thus high prevalence of sub-clinical vascular disease (Radford & Church 2002). Both trials have been criticised for the relatively high doses of hormones used and particularly the synthetic progestin, medoxyprogesterone, since this hormone can inhibit the beneficial effects of oestrogen (Pearson Murphy 2002). These recent randomised control trials are disappointing and surprising, but it should be noted that hormone replacement therapy does not replicate the same physiology as seen in the pre-menopausal woman. Although female HRT cannot currently be recommended to reduce CAD risk it may well be that different hormone preparations and delivery in a younger population will have some efficacy.

In comparison, the role of androgens in CAD has been relatively neglected, although a detrimental effect is usually presumed. However, we have found that low rather than high testosterone levels are associated with CAD (English et al. 2000a) and that low serum testosterone is associated with increased aortic atheroma (Hak et al. 2002). Furthermore, low testosterone levels are associated with several risk factors for the development of CAD,

**Figure 1** Immune and inflammatory reaction. T lymphocytes and monocytes are drawn to the plaque and pass through the endothelium. T-cells interact with antigens and other immune cells producing proinflammatory cytokines. Monocytes mature into macrophages producing cytokines and collagens. There is self-perpetuation with the production of chemoattractants such as MCP-1 and expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1). IFN, interferon; oxLDL, oxidised LDL; MMPs, matrix metalloproteinase enzymes.
including systolic and diastolic hypertension, adverse lipid profile and high levels of fibrinogen, insulin and pro-coagulable factors (reviewed in English et al. 1997).

Despite this, it has yet to be established that low testosterone level is an independent risk factor for the development of CAD in man; it may simply be an epiphenomenon. There is, however, experimental evidence that androgens have a disease-modifying role in atheroma formation. Several male animal studies have shown that androgens reduce diet- and injury-induced atherosclerosis. These studies have their limitations, as most use the cholesterol-fed rabbit with or without induced endothelial injury. This is a well-established model but has some differences compared with native human atheroma. The cholesterol diet creates a hyper-lipidaemia characterised by high very low-density-lipoprotein (VLDL) rather than a high LDL more commonly seen in humans and the lipid deposition seen is in the form of cholesterol ester-enriched fatty streaks rather than mature plaque (Arad et al. 1989). However fatty streaks are known precursors of mature plaque and the morphology and behaviour of cells in the vessels of animal model fatty streaks mimics that seen in human atheroma (Gordon et al. 1988). The results are tabulated in Table 2.

All but one study has shown a beneficial or neutral effect of androgen therapy on the formation and progression of atherosclerosis. The only negative study, by Toda et al. (1984), used a dose of testosterone at 150 mg per day.

Figure 2 (A) Stable plaque characterised by small necrotic core (NC), abundant vascular smooth muscle cells, and a dense thick plaque cap with extensive collagen/elastin fibres. (B) Unstable plaque characterised by a larger necrotic core, thin plaque cap with sparse smooth muscle cells and collagen fibres, inflammatory cells producing cytokines and MMPs and apoptic/senescent smooth muscle cells.
which is a very high dose and caused increases in fatty accumulation in the aorta (as well as delayed maturation of external phenotype) whereas a lower 30 mg dose showed no increase in fatty accumulation. The other studies use doses of hormones that create levels either within the physiological range or modestly supra-physiological. The study of Alexandersen et al. (1999) is the most elegant model. Here, castration of male animals without hormone replacement induced a 100% increase in aortic atheroma compared with sham-operated animals. Testosterone replacement to a high physiological range or intra-muscular injections or tablets inhibited this response. The final amount of aortic atheroma was less in the intramuscular testosterone group than in the sham-operated group. The reason for this is not certain but the final fasting testosterone level in the intramuscular testosterone group than in the sham-operated group. The means by which testosterone may confer benefit in CAD is not clear. We have previously demonstrated that 3 months of physiological testosterone treatment leads to symptomatic improvement in men with angina (English et al. 2000b). The acute and chronic anti-Ischaemic properties of testosterone have been reported by other groups (Jaffe 1977, Rosano et al. 1999) and this effect is most likely mediated by coronary artery vasodilatation (Webb et al. 1999), which appears to involve calcium channel antagonism in a gender-specific fashion (English et al. 2001, 2002). However, we propose that testosterone may also have a role in limiting the vascular inflammation and cytokine activity underpinning the pathophysiology of atherosclerosis. Macrophages, lymphocytes and vascular smooth muscle cells (VSMC) all possess androgen receptors (Fujimoto et al. 1994, Benten et al. 1999a,b). In addition, there is evidence of a biofeedback loop since cytokines appear to impair synthesis and release of testosterone (Mealy et al. 1990). There have been no studies directly examining the action of androgens on cytokine production in patients with CAD. However, there is evidence from several studies that androgens possess immune-modulating properties. In the majority of these reports, androgens, including testosterone, have been shown to suppress the activity of pro-inflammatory cytokines while enhancing that of anti-inflammatory cytokines (see Table 3). In a recent study, hypogonadal men were found to have a greater degree of inflammatory activation compared with healthy controls, including serum cytokine levels (Yesilova et al. 2000). Androgen therapy in these patients led to a reduction in circulating cytokines. Clearly these studies only provide circumstantial evidence of an anti-cytokine effect of testosterone and further studies are needed. It is of interest that testosterone has been used with some success to treat males with autoimmune rheumatic conditions (Bizzarro et al. 1991). Although the studies do not report levels of serum cytokines, these diseases are essentially cytokine mediated and clinical improvement would be expected to be associated with reduced expression of pro-inflammatory cytokines.

In addition to direct anti-inflammatory actions, testosterone also appears to have an important effect on rates of vascular smooth muscle cell proliferation and apoptosis, an important factor in maintaining plaque integrity (Newby & Zaltsman 1999). The anabolic actions of testosterone in increasing protein synthesis and skeletal muscle cell size via Testosterone and cytokines – a possible mechanism of protection

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nuclear transcription are well documented. Testosterone has been shown to reverse castration-induced skeletal muscle apoptosis in rats (Boissonneault 2001) and inhibit human neuronal apoptosis (Hammond et al. 2001). Importantly, it has also been shown to produce proliferation of human vascular smooth muscle cells (Williams et al. 2002). Testosterone could, therefore, potentially be involved in maintaining the fibrous cap of the atherosclerotic plaque by promoting smooth muscle cell stability.

**Conclusion**

Cytokine activation and vascular smooth muscle cell apoptosis play an integral part in the development of CAD and in the pathophysiology of acute coronary syndromes. Experimental studies show that androgens protect against the development of atheroma. Separately, androgens have been shown to suppress pro-inflammatory cytokine activity, inhibit apoptosis and enhance vascular smooth muscle cell proliferation. These actions may be responsible for their athero-protective effects. To test this hypothesis, further studies are needed, including clinical trials, to examine this mechanism and the role of androgens in cardiovascular disease.

**References**


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Testosterone protection against atherosclerosis


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