Prospective Study of Effect of Androgens on Serum Inflammatory Markers in Men

Martin K.C. Ng, Peter Y. Liu, Andrew J. Williams, Shirley Nakhla, Lam P. Ly, David J. Handelsman, David S. Celermajer

Objective—Because male sex is an independent risk factor for the severity of atherosclerosis, it is possible that androgens may be proatherogenic. There is evidence that sex hormones, particularly estrogens, regulate (or modulate) inflammation, a process integral to atherogenesis. Because levels of serum inflammatory markers predict cardiovascular outcomes, we prospectively assessed the effects of androgen therapy on these markers in older men.

Methods and Results—Levels of high-sensitivity C-reactive protein (CRP), soluble intracellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) were measured from sera collected at baseline and at the end of 2 randomized double-blind placebo-controlled trials evaluating the effects of 3 months of androgen treatment with either dihydrotestosterone (DHT) or recombinant human chorionic gonadotropin (rhCG) in healthy men aged >60 years with partial androgen deficiency (serum testosterone levels <15 nmol/L). For the DHT study (70 mg transdermally daily), 33 men completed 3 months of treatment (16 men were treated with DHT, and there were 17 controls). For the rhCG (250 µg twice weekly) study, 20 men were treated with rhCG, and there were 20 controls. In both studies, groups were well matched for age and vascular risk factors. Androgen levels (DHT and testosterone) were consistently maintained at eugonadal levels throughout the trials, with estradiol markedly increased by rhCG but not DHT. Baseline CRP levels were 0.74 to 1.49 mg/L, sVCAM-1 levels were 847 to 950 ng/mL, and sICAM-1 levels were 256 to 292 ng/mL in all groups. Neither DHT nor rhCG resulted in significant changes in CRP, sVCAM-1, or sICAM-1 compared with placebo (P>0.3 in both studies).

Conclusions—Exogenous androgen therapy with or without increased estradiol levels does not alter serum inflammatory markers in older men; this finding is in contrast to the effects of estrogens on inflammatory markers that have been found in postmenopausal women. These data provide a measure of reassurance concerning potential adverse cardiovascular effects of androgen therapy in older men. (Arterioscler Thromb Vasc Biol. 2002;22:1136-1141.)

Key Words: inflammation ♦ testosterone ♦ estrogen ♦ atherosclerosis

The potential for the use of androgen therapy in older men with partial androgen deficiency to improve the quality of life and muscle strength is being increasingly discussed.1 Because men have more severe and extensive coronary disease than do age-matched women in almost all populations,2,3 it is possible that androgens may actually be proatherogenic. At present, however, the effects of androgen replacement therapy on the cardiovascular system of older men are virtually unknown.4

There is increasing evidence for the role of inflammation in promoting atherogenic risk.5 Elevated C-reactive protein (CRP) levels have been shown to predict adverse cardiovascular outcomes in men and women.6,7 The binding and recruitment of circulating monocytes to the vascular endothelium, key processes in early atherogenesis, are mediated by a family of cellular mediators of inflammation or cell adhesion molecules (CAMs), including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Elevated concentrations of soluble ICAM-1 predict the risk of myocardial infarction in men.8 Furthermore, high circulating levels of VCAM-1 have been associated with increased carotid intimal-medial thickness (a marker of subclinical atherosclerosis),9 with the extent of peripheral vascular disease as evaluated by angiography,10 and with future risk of cardiovascular death in patients with angiographically documented coronary artery disease.11

Sex hormones may play a role in the regulation of inflammatory responses. For example, estrogen-based hormone replacement therapy (HRT) increases CRP levels in postmenopausal women.12-14 In contrast, HRT reduces circulating levels of cell adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin.15-18 The effects of exogenous
androgens on inflammatory markers have not been examined. We have previously shown that androgens increase endothelial VCAM-1 expression in vitro, and we now report the effects of androgen treatment on serum inflammatory markers in healthy older men.

Methods

Description of Studies

Two separate, 3-month, randomized, double-blind, placebo-controlled clinical trials were conducted; each trial compared the effects of a different androgen therapy with placebo on muscle strength, functional measures (including spontaneous physical activity), and quality of life in partially androgen-deficient older men. One study evaluated the effects of dihydrotestosterone (DHT, an androgen not capable of aromatization). DHT (70 mg daily) was administered via a transdermal gel (Laboratoires Besins-Iscovesco). The design and main results of that study have been published. A second trial evaluated the effects of recombinant human chorionic gonadotropin (rhCG) administered 250 μg SC twice weekly (Se- nore, rhCG is a placental hormone that is a close analogue of pituitary luteinizing hormone and acts on the same Leydig cell receptor to increase testosterone production and, thereby, estradiol formation via the aromatization of testosterone.

In the DHT study, at randomization, 18 men were assigned to DHT and 19 men were assigned to placebo, with 17 and 16 men, respectively, completing the study. Of the 4 subjects who discontinued after randomization, 2 (both on placebo) were lost to follow-up, 1 (on DHT) discontinued because of dislike of the gel, and 1 (on placebo) discontinued because of apparent worsening of his arthritis. In the rhCG study, at randomization, 20 men were assigned to rhCG, and 20 men were assigned to placebo. All enrolled men completed the rhCG study. One 3-month serum sample (of a patient assigned to rhCG) was lost and was therefore unavailable for analysis.

Subjects

The DHT and rhCG studies included healthy ambulatory men aged >60 years with partial androgen deficiency, defined as the presence on 2 occasions of low serum total testosterone (<15 nmol/L). Men were excluded from the studies if they had prostatic disease requiring medical or surgical treatment or significant chronic medical diseases likely to interfere with safe participation (including advanced chronic renal or liver disease, unstable chronic pulmonary or cardiovascular disease, uncontrollable or severe hypertension, hyperlipidemia, obstructive sleep apnea, polycythemia, or malignancy with poor prognosis). Patients were also excluded from the study if they were taking medications that were likely to interfere with the evaluation of the study end points; these medications included androgens or other sex steroids, antiandrogens, cimetidine, spironolactone, and gonadotrophin-releasing hormone analogues. Both studies were approved by the institutional review committee, and participants provided written informed consent in accordance with approved guidelines.

Blood Collection and Laboratory Assays

Fasting morning blood samples were obtained from each patient at baseline and at 3 months after randomization. Serum was separated by centrifugation and then stored at −20°C. High sensitivity CRP (hs-CRP) was measured by rate nephelometry on an Immage automated nephelometer (Beckman Coulter). This hs-CRP assay has been in use for many years in diagnostic laboratories and correlates well with other commonly used assays. In addition, we have previously analyzed 270 blood bank donors with the use of this method and achieved a reference range similar to those published in the literature. Briefly, 20 μL of sample was added to 20 μL of particle-bound CRP antibody complex and incubated for 3 minutes, after which the rate of light scatter caused by the formation of CRP–particle-bound CRP antibody complex was measured and compared with a standard curve constructed from standards with known amounts of CRP. This hs-CRP assay has a functional sensitivity of 0.15 mg/L with a coefficient of variation (CV) of 9.1% at this level. Within-run precision was 1.7% for CRP of 0.15 to 3 mg/L. Serum levels of sICAM-1 and sVCAM-1 were measured by ELISA (Chemicon), with respective CVs of 4.1% and 5%. ELISAs for circulating cell adhesion molecules (ICAM-1 and VCAM-1) were “batch-processed” at the end of the study. That is, every patient’s baseline sample and 3-month serum sample for each CAM were processed at the same time by a single operator blinded to the study protocol.

Hormones were measured by methods as described previously. Briefly, total testosterone was measured by commercial immunoassay (Immulyte, CV 7.8% to 12.7%). DHT was measured by the permanganate method with the use of a testosterone antibody (C0457, Bioquant; CV 3.8% to 4.6%). Estradiol was measured in unextracted plasma samples by use of a DELFIA assay (Perkin-Elmer Corp, CV 1.2% to 5.8%). Samples were handled in an identical and blinded fashion throughout the study and were measured within a single assay as far as possible.

Statistical Analysis

SPSS (version 9) was used for statistical analysis. Analyses were limited to men for whom baseline and 3-month serum samples were available. Baseline characteristics, including values for the inflammatory factors, were compared between randomized treatment groups with the use of t tests for independent samples, with the Hochberg modification of the Bonferroni procedure to account for multiple comparisons. Mean 3-month levels and mean change from baseline levels of serum inflammatory markers between active and placebo treatment arms of each study were compared by using t tests for independent samples. Because distributions of CRP were skewed, CRP values were logarithmically transformed before the calculations (these transformed values were normally distributed, as tested by Lilliefors modification of the Kolmogorov-Smirnov test).

Given the baseline data from both studies, each had >80% power to detect an androgen-related difference in inflammatory marker levels of 1 SD between groups at the 2P<0.05 level.

Regarding study power, it is noteworthy that in previous trials examining hormone therapy and inflammatory markers, effect sizes of at least 1 SD have been observed. For example, Cushman et al found that estrogen only and estrogen/progestin therapy were both associated with an increase in CRP levels of ≥1 SD compared with baseline, and Caulein-Glauser et al found that hormone replacement compared with no treatment was associated with a change in ICAM-1 levels of ≥3 SDs. Therefore, the present study was powered to detect even a relatively modest androgen-related change in inflammatory marker levels compared with effects previously observed in the sex steroid studies reported to date.

Results

In both studies, groups as defined by treatment assignment were well matched for age and vascular risk factors (Table 1).

DHT Study

Baseline median CRP levels were 1.41 and 1.49 mg/L, mean sICAM-1 levels were 256 and 264 ng/mL, and mean sVCAM-1 levels were 859 and 950 ng/mL in placebo- and DHT-assigned treatment groups, respectively. These were well matched between treatment groups (Table 1). Baseline levels of sex hormones were also similar among placebo- and DHT-treated groups (Table 2). In patients assigned to androgen treatment, DHT levels increased during treatment, and this was accompanied by a fall in total testosterone relative to placebo-assigned patients (Table 2). Estradiol levels were not affected by DHT treatment (Table 2).

After 3 months of therapy, median CRP levels were 1.65 and 1.50 mg/L, mean sICAM-1 levels were 228 and 224
ng/mL, and mean sVCAM-1 levels were 861 and 895 ng/mL in placebo- and DHT-assigned treatment groups, respectively. DHT treatment had no significant effect on either CRP, sVCAM-1, or sICAM-1 compared with placebo (P > 0.4 versus control for all serum inflammatory markers; Figure, panel A).

rhCG Study
Baseline levels of serum inflammatory markers were well matched between placebo- and rhCG-treated subjects (Table 1). Median CRP levels were 0.74 and 1.07 mg/L, mean sICAM-1 levels were 292 and 268 ng/mL, and mean sVCAM-1 levels were 847 and 896 ng/mL in placebo- and rhCG-assigned treatment groups, respectively (Table 1). Baseline levels of estradiol and testosterone were also similar among placebo- and rhCG-treated groups (Table 2). As expected, rhCG therapy produced significant increases in serum testosterone and estradiol levels (Table 2).

After 3 months, rhCG had no significant effect on either CRP, sVCAM-1, or sICAM-1 compared with placebo (median CRP levels were 0.85 and 1.05 mg/L, mean sICAM-1 levels were 295 and 268 ng/mL, and mean sVCAM-1 levels were 295 and 268 ng/mL, and mean sVCAM-1 levels were 847 and 896 ng/mL in placebo- and DHT-assigned treatment groups, respectively; P > 0.3 versus control for all serum inflammatory markers; Figure, panel B).

**Discussion**
Androgen replacement in older men to eugonadal levels for 3 months had no significant effects on the serum levels of CRP, sICAM-1, or sVCAM-1. In this context, an increase in serum estradiol in older men also had no significant effect on serum inflammatory markers; this finding is in contrast to the effects exerted by estrogens on serum inflammatory markers in postmenopausal women. Each study had >80% power to detect even relatively modest changes (1 SD of the baseline percentile value, 75th percentile value).

There were no significant differences among treatment arms for any of the variables shown.

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<tr>
<th>TABLE 2. Baseline Levels of Sex Hormones by Treatment Assignment</th>
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<td><strong>Time, Months</strong></td>
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Values are presented as mean±SD. NM indicates not measured.
that acts on luteinizing hormone/human chorionic gonadotropin receptors to increase testosterone production by Leydig cells in the testes. In the body, testosterone is partially metabolized by aromatase to estradiol and by 5α-reductase to DHT. Therefore, treatment with rhCG produces increases in serum testosterone and estradiol levels. DHT, by comparison, is an androgen not capable aromatization and, therefore, does not produce any estrogenic effects. Thus, these 2 randomized prospective studies provided an ideal opportunity to study the effects of androgens and estrogens on serum inflammatory markers in older men.

We have previously reported that androgen exposure at supraphysiological levels (DHT at concentrations of at least 40 nmol/L) increases VCAM-1 expression in human endothelial cells in vitro. The apparent lack of correlation between the current clinical data and data from our in vitro study is not surprising given the broad cellular distribution of the CAMs. An increase in CAM expression by a specific cellular subpopulation may not be detectable in the serum, where the measured level is the result of proteolytic cleavage of CAMs from the surface of a variety of cell types. Also, inasmuch as our 2 studies have evaluated the effects of androgen replacement to physiological or mildly supraphysiological levels only, it is possible that effects of androgens on circulating VCAM-1 might only occur at concentrations considerably above those achieved in the present study.
The neutral effect of administration of a pure androgen (DHT) and of combined androgen and estrogen therapy (rhCG) on serum inflammatory markers in older men contrasts with the effects of HRT in postmenopausal women. Recent studies have reported that opposed (by progesterone) and unopposed estrogen replacement therapy decrease levels of circulating ICAM-1, VCAM-1, and E-selectin in postmenopausal women. In a cross-sectional study, Oger et al reported that the reduction in circulating ICAM-1 due to transdermal estrogen therapy was dependent on treatment duration, with long-term use being necessary for the effect to be observed. No such time-dependent effect was observed with oral estrogen replacement.

Recent cross-sectional and prospective studies have also reported that estrogen HRT produces a sustained increase in CRP levels in postmenopausal women. Given that elevated CRP levels are an independent predictor of future cardiovascular risk in such women, it has been hypothesized that HRT may have a proinflammatory effect that may contribute to atherosclerotic plaque instability and thrombosis. This hypothesis has been given some support by a report of an increase in cardiovascular events among women assigned to HRT in the first year after randomization in the Heart and Estrogen/Progesterin Replacement Study (HERS).

Our finding of a neutral effect of rhCG administration on serum inflammatory markers in older men is interesting. In view of the absence of an effect on inflammatory markers with DHT, a pure androgen, results from our rhCG study suggest that estrogens may not have a significant effect on circulating ICAM-1, VCAM-1, or CRP in older men. Our observations suggest that a sex difference may exist regarding the effects of estrogens on serum inflammatory markers. Such a phenomenon would be consistent with our previous observations of sex-specific effects of estrogen, progesterone, and androgens on human macrophage foam cell formation and may hold implications for the sex difference in atherosclerosis.

Limitations of the present study warrant consideration. Because of the study design (androgen replacement in older men), the study population consisted of men aged >60 years, subjects who probably had a high prevalence of asymptomatic atherosclerosis. Different effects may be seen in younger men, with a lower prevalence of disease. Men were included in the present study if they had a baseline serum total testosterone level of <15 nmol/L (which has been termed partial age-related androgen deficiency). Nevertheless, this group of subjects appear quite representative of older men in general, on the basis of the testosterone levels and inflammatory marker values measured, compared with previously studied normal populations. For example, 68% of all recruited subjects in the present study had testosterone levels within the normal range (11 to 35 nmol/L). At least 30% of all community-dwelling men aged >60 years have serum testosterone levels <15 nmol/L. In addition, baseline levels of circulating ICAM-1, VCAM-1, and CRP in our cohort of older men are similar to previously published data. For example, the median baseline CRP level in our study population was 1.3 mg/L, identical to the median CRP level in 1172 apparently healthy men aged 40 to 84 years participating in the Physician’s Health Study. Likewise, baseline circulating ICAM-1 and VCAM-1 levels in all treatment groups were within the range of values published for middle-aged to elderly men with or without atherosclerosis.

Hence, the results of the present study appear to be applicable to a large proportion of similarly aged men. Finally, our finding of a lack of increase in CRP or decrease in CAMs after an increase in endogenous estrogen is difficult to interpret biologically, because androgen and estrogen levels were altered by rhCG administration. Therefore, this finding may be more relevant to the clinical setting (of androgen replacement) than to the effects of estrogen administration, per se.

In summary, in these 2 randomized prospective trials, the administration of androgens (with or without concomitant increase in estradiol levels) for 3 months in apparently healthy older men with partial androgen deficiency had no significant effects on the levels of the important inflammatory markers ICAM-1, VCAM-1, and CRP. These results contrast with in vitro findings for androgens and the significant effects of estrogen replacement on inflammatory markers in postmenopausal women. Furthermore, these data provide a measure of reassurance concerning the effects of androgen administration to older men.

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References


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