Possible Interactive Effect of Testosterone and Aldosterone Receptor Antagonists on Cardiac Apoptosis
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Chronic heart failure (CHF) is a major public health problem, with rising clinical, social, and economic burden. Although the etiology of heart failure (HF) involves multiple agents and conditions, several data show that cardiomyocytes apoptosis is increased in failing hearts thus suggesting that programmed cell death (apoptosis) may be one of the most important mechanisms favoring the progression of HF. Cardiomyocytes apoptosis contributing to the loss of cardiac cells is involved in the progression of HF of either ischemic or non-ischemic origin.1,2

Increasing evidence also suggests that a variety of hormones, including sex hormones, may be down-regulated in cardiovascular disease and HF.3,4 Among these, androgens are associated with important and multifaceted effects on the heart and the vascular system in both sexes. In particular testosterone influences cardiac and vascular function, affecting endothelial function, vascular tone and cardiac function. Testosterone influences the progression of atherosclerosis and has extra-cardiac effects that are important for the functional capacity of patients with HF. Animal studies suggested that low testosterone levels favour the development of coronary atherosclerosis in male animals, similarly studies in human have also reported that low testosterone levels in men are independently associated with the development of atherosclerosis and with an increased risk of future cardiovascular events.5,9

A depletion in the plasma levels of testosterone is a relatively common finding in patients with HF and represents one of the most important factors contributing to the shift of the anabolic/catabolic balance, towards catabolism, which is often observed in patients with advanced HF.3 Reduced testosterone levels predict a negative prognosis in patients with HF and testosterone supplementation, within the physiological range, has acute and long-term beneficial effect in patients with HF.9 Studies in patients with HF have shown that androgen supplementation with physiologic doses of transdermal testosterone improves symptoms, exercise capacity and quality of life in patients with HF.10,11

However, while some hints have been suggested to explain the effect of testosterone on the progression of atherosclerosis, little is known about the mechanism underlying the cardio-protective effects of androgens in HF, especially with regards to their effect on renin-angiotensin-aldosterone system and cardiomyocytes and skeletal muscle apoptosis.

The study published in this issue of Revista Española de Cardiología by Sánchez-Más et al12 was designed to address whether testosterone reduces cardiomyocytes apoptosis, and whether the concomitant administration of spironolactone or eplerenone, affected the effects of testosterone. The study also addressed the question whether the effect of testosterone on cardiomyocytes apoptosis was mediated by androgen receptor (AR) by the co-administration of testosterone and flutamide, a potent androgen receptor antagonist.

Apoptosis was induced using a hyperosmotic stress with sorbitol and assessed as cell viability, DNA fragmentation and caspase-3, -8, and -9 activation. Testosterone significantly reduced the sorbitol-induced apoptosis, as shown by the increment in cell viability and the reduction in the caspases activation. Of interest the addition of flutamide, did not alter the protective effect of testosterone on cell viability, whilst it further increased the inhibition of caspases mediated by testosterone.

Sánchez-Más et al12 explained this discrepancy suggesting a potential non-genomic effect of testosterone, in addition to the classical genomic signaling. However, these divergent effects of AR blockade on 2 related end-points (cell viability and caspase activation) should be better elucidated.
The rapid, non-genomic, effects of androgens may be mediated by a direct influence of ion-channels and transporters (e.g., Ca\(^{2+}\), Na\(^{+}\), K\(^{-}\)) or through an indirect modulation by induction of conventional second messenger signal transduction cascades, including increases in free intracellular Ca\(^{2+}\), activation of protein kinases A/C (PKA/PKC) or mitogen-activated protein kinase (MAPK).\(^{13}\) In order to support the hypothesis of a rapid, non-genomic effect of testosterone, the authors evaluated the role of the signaling pathways SAPK/JNK, ERK 1/2, and p-38 MAPK.

However, caution is needed to interpret the results, in experiments using testosterone, because its effects may differ when applied acutely or chronically. Since cells were exposed for 3 hours to ligands, the biological effect of testosterone may be in contrast with the hypothesis of a rapid, non-genomic, effects that is known to occur within seconds or minutes.

Another issue raised by the study is the potential interference of mineralocorticoid system in the testosterone-mediated effect on apoptosis. To this aim, the authors pre-treated cardiomyocytes with 2 different mineralocorticoid blockers, spironolactone or eplerenone, showing that spironolactone blocked the effects of testosterone, reducing cell viability and increasing the activation of caspases 3, 8, and 9, while eplerenone increased cell viability but did not affect the decrease of caspases activation induced by testosterone. This diverging effect may be due to the different molecular properties of the two mineralocorticoid receptor (MR) blockers. As known, spironolactone is an antimineralocorticoid associated with progestogenic and anti-androgenic effects, while eplerenone is a derivative of spironolactone with a higher selectivity to the MR.

The authors have hypothesized that the different effects of the 2 MR blockers may be due to the progestogenic effects present in spironolactone and absent in eplerenone, and not to the different antagonistic potency of the drugs on the MR. However, the effects of aldosterone in this cellular setting has not been studied, and this would have been a critical step to assess a potential role of MR in affecting apoptosis in cardiomyocytes. Aldosterone has emerged as a key hormone determining cardiovascular and renal damage and risk of future events, in addition to its role in blood pressure regulation and potassium and sodium homeostasis. The MR is a promiscuous receptor and has not only high affinity for aldosterone but it possesses also high affinity for glucocorticoid hormones; in particular, its affinity for cortisol is >10-fold higher than that of the glucocorticoid receptor.\(^{14}\) Mineralocorticoid receptors are also expressed in non-epithelial tissues, including the cardiovascular and central nervous systems and adipose tissue. In these tissues, glucocorticoids might represent the predominant endogenous ligand, given the absence of significant 1\(\beta\) hydroxysteroid dehydrogenase type 2 (11HSD2) activities.\(^{15}\) Furthermore, increasing evidence from animal studies support the hypothesis that a change in the redox state of the cell can increase MR signalling mediated by glucocorticoids.

The lack of insights in the potential role of testosterone conversion into estrogens is an unanswered question arising from this study. Indeed, there is substantial evidence that physiological testosterone levels have a beneficial effect on blood vessels and cardiovascular system and that this is due to conversion of testosterone to estrogen. The aromatization of testosterone to estradiol is a critical mechanism involved in the protective effects of testosterone on atherogenesis in male animals, as shown by the fact that aromatase inhibition in male mice increases fatty streaks by the same extent of castration, and that the aromatizable androgen DHEA (dehydro-epiandrosterone) inhibits atherosclerosis in intact cholesterol-fed rabbits, whereas non-aromatizable androgens have no effects.\(^{6}\)

Although the treatment of HF has made significant advances in the past decades, the prognosis of the affected patients remains poor, the development of new therapeutic approaches is needed and mineralocorticoid antagonism and treatment with testosterone are amongst the interventions that have shown positive effect in HF. The findings of the present study may have significant clinical implications as they suggest an interplay between testosterone and mineralocorticoid blockade on apoptosis in HF. In conclusion, this study showed differences in biological effects of spironolactone and eplerenone on the action of testosterone at the level of the cardiomyocytes, suggesting that while the benefits of spironolactone on the cardiomyocytes may be reduced by blocking the protective effect of testosterone, those of eplerenone may have an advantage with respect to spironolactone, since it does not diminish the anti-apoptotic effect of testosterone. The diverging effects of the two molecules should be further investigated by studying the role of progesterone receptor in mediating the potential progestogenic effects of spironolactone. Therefore, this study provides speculations about a potential therapeutic role of the concomitant use of testosterone and eplerenone in HF patients.

REFERENCES