Original article

Non-expression of androgen receptors in the carotid intimal medial zone

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Abstract

Androgens and oestrogens have been implicated in the noted gender differences in the increasing intimal medial thickness. Oestrogens are protective in females while the role of androgens as male disadvantage remains in conflict. There have been reports that androgens are protective while other reports suggest they are bane. The distribution of androgen receptors in the carotid intimal medial thickness may help explain this propensity. Thirty six samples from the proximal, middle and distal carotid artery segments and three sections of prostate gland from three different men were collected within 48 hours of demise were processed for routine light microscopy and immunohistochemistry. The prostate samples were mounted next to the carotid artery samples on the same slide for immunohistochemical staining. They were stained using anti-human mouse androgen receptor monoclonal antibody (AR318) for androgen receptors. All prostate control samples stained for androgen receptors. Androgen receptors were not expressed in any of the carotid arterial walls. The carotid intimomedial thickness is not influenced by the presence of androgen receptors.

Keywords: androgen receptors, carotid intimal medial thickness.

1 Introduction

Carotid intima-medial thickness (CIMT) provides a valuable tool for physicians to evaluate the cardiovascular disease risk profile of their patients (COBBLE and BALE, 2010; JARAUTA, MATEO-GALLEGO, BEA et al., 2010). It is a predictor of atherosclerosis and its correlations with cardiovascular morbidity has been firmly established (HODIS, MACK and LABREE, 1998; BOTS, HOES, KOUDSTAAL et al., 1997; O'LEARY, POLAK, KRONMAL et al., 1999). CIMT is worse in males than in females and increases with age with this gender gap narrowing after the menopause (JARAUTA, MATEO-GALLEGO, BEA et al., 2010; LERNER and KANNEL, 1986; OSIKA, DANGARDT, MONTGOMERY et al., 2009). The gender difference in structure of the carotid artery and subsequent development of atherosclerosis has been related to the effects of androgens (RAMIREZ, AZCONA, BLASCO et al., 2007; PARCHAMI and DEHKORDI, 2011). It is known that males are prone to early development of carotid atherosclerosis (WU and VON ECKARDSTEIN, 2003). In comparison, the low incidence of atherosclerosis in the premenopausal women has supported the theory of 'beneficial estrogen' and 'harmful androgens' (TRACY, 1966; LIU, DEATH and HANDELSMAN, 2003; PHILLIPS, 2005). Studies have also shown a direct relationship between the testosterone levels in men with the increased CIMT, progression of atherosclerosis, accumulation of visceral adipose tissue, and other risk factors for carotid atherosclerosis (RAMIREZ, AZCONA, BLASCO et al., 2007; MALKIN, PUGH, JONES et al., 2003). On the contrary, high levels of androgens in men, and in some women with high dihydroepindrosterone (DHEAS) polycystic ovarian syndrome (PCOS) have also been associated with a lower CIMT when other risk factors are controlled (BERNINI, SGRO, MORETTI et al., 1999; MEYER, McGRATH, CAMERON et al., 2005; VRYONIDOU, PAPATHEODOROU, TAVRIDOU et al., 2005). The exact role of androgens in development and progression of atherosclerosis is therefore controversial; with some studies showing protection (BONNEL, PRITCHETT and RARDIN, 1941; ALEXANDERSEN, HAARBO, BYRJALSEN et al., 1999) while others demonstrate detriment (RAMIREZ, AZCONA, BLASCO et al., 2007). Vascular androgen receptors (AR) may have a cellular or zonal preference and their expression may be related to the general structure of the artery, especially the intimal medial thickness. This information could predict the role these receptors in increased intimal medial thickness and subsequent development of atherosclerosis.

2 Samples and methodology

Thirty six segments from twelve (6 males and 6 females) carotid arteries were harvested within 24 hours of demise and fixed in 10% formaldehyde. Three post-mortem adenocarcinomatous prostates from 3 different males were harvested within 48hrs of demise were used as positive controls for androgen receptor immunohistochemical staining in this study (BAYER-GARNER, GIVENS and SMOLLER, 1999; ROCHA, WICKHAM, DA SILVERA et al., 2000). The omission of the primary antibody and substitution with dilution solution alone served as a negative control (GALLARDO, LLORETA, GARCIA et al., 2009). An ethical approval had been sought from the Kenyatta National Hospital/University of Nairobi ethical research committee. The tissue segments were processed and blocked for routine immunohistochemistry. Paraffin sections 3 μm from the blocks above were cut and mounted on previously charged microscope slides. These included a positive control (prostate sample) and the test section (carotid sample). The sections were fixed in the bond-max covertiles. Respective labels were then affixed to the slides and the racks slotted into the machine for autostaining. Staining protocol F was

used. This is a high amplification, biotin free detection system optimized for use on the bond system. Autoimmunostaining occurred as follows; dewaxing was done by a commercially available dewax solution® for 15 minutes. Specimens were incubated with hydrogen peroxide for 5 minutes to quench endogenous peroxidase activity. Antigen retrieval was done by microwaves at 98 °C for 20 minutes at a Ph of 9.0 (JANSSEN, BRINKMANN, BOERSMA et al., 1994). They were then incubated with AR – 318 the primary antibody for 15 minutes then rinsed using bond wash buffer (SAJJAD, QUENBY, NICKSON et al., 2004). Post primary IgG linker reagent was applied to the sections for 8 minutes to localize the antibody, followed by a buffer wash. Sections were then incubated with bond polymer for 8 minutes then rinsed using bond wash buffer. Sections were incubated for 10 minutes in 3,3'-diaminobenzidine tetra hydrochloride (DAB) (Sigma) which forms a brown precipitate with the complex so as to aid visualization. Harris haematoxylin counterstaining was done for 5 minutes to allow visualization of the nuclei (Merck, Poole, Dorset, UK). At the end of the staining the racks were dislodged from the staining chamber and the stained slides fitted into a staining rack. The sections were dehydrated into two changes of alcohol, and cleared in three changes of xylene. The slides were then mounted in DPX, ready for microscopy. Slides were examined by two independent observers who were unaware of the gender and ages of the individuals. The total cell count and the number of stained cells for each designated vessel zone were assed in the three sections in three to four visual fields per section at 400× magnification using a Zeiss® photomicroscope.

3 Results

Thirty six vessel segments from six males and six females were assessed. Three prostate samples were used as controls. All prostate samples expressed androgen receptors. Carotid artery displayed nuclei for the different mural cells. None of the cells in the different carotid artery samples or segments stained for androgen receptors (Figure 1).

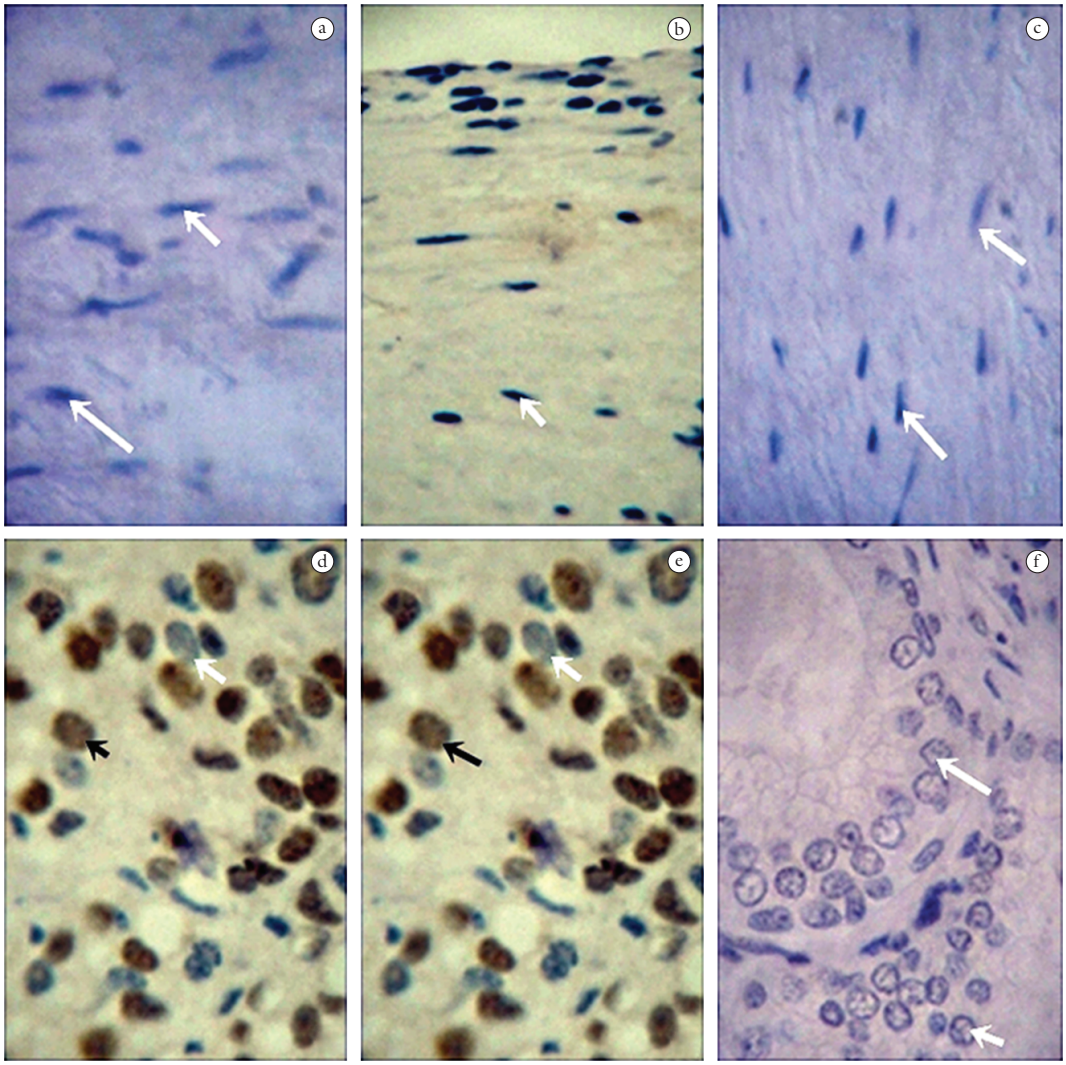

Figure 1. Non expression of androgen receptors in the carotid arterial wall. Photomicrographs showing the connective tissue cell nuclei (purple staining) and androgen receptors (brown staining). Images (a), (b) and (c) are sections of proximal, middle and distal carotid segments from a 12 years old female, 30 years old male and 71 years old male respectively. The non-AR staining cells are labelled with white arrows. Images (d) and (e) are prostate samples with the brown (black arrowed) immunopositive AR cells. Image (f) shows a prostate deprived of the marker, used as a negative control.

4 Discussion

Androgen receptors were not expressed in any of the layers, segments or age groups of the common carotid artery. Findings of the present study are at variance with the observations of Liu, Christian, Ruan et al. (2005) who immunolocalised androgen receptors in the post-mortem coronary arterial wall, inversely relating their numbers to early atherosclerosis. The absence of these receptors in the carotid could be normal, observed in other normal body tissues such as thyroid, pancreatic, gastrointestinal and bladder tissues, which do not express AR (DE WINTER, TRAPMAN, VERMEY et al., 1991). The absence of these receptors in the carotid wall suggests that androgen receptors have a limited genomic role in the carotid arterial structure and subsequent development of atherosclerosis. A similar conclusion was made by Christian, Liu, Harrington et al. (2006), who did not localise AR in the coronary arterial wall. On the contrary, McRobb, Handelsman and Heather (2009) immunolocalised AR in the rat innominate artery and the aortic sinus and positively associated them with increased calcification of the atherosclerotic plaques. The absence of this receptor in the CCA could be partly attributed to post-mortem loss or low numbers of AR limiting detection (MAINWARING and MANGAN, 1973). Fodor, Van Leeuwen and Swaab (2002) found that the post-mortem stay before fixation and the duration of formalin fixation affects the expression of steroid receptors in post-mortem material. This receptors can however be retrieved by microwave extraction (FODOR, VAN LEEUWEN and SWAAB, 2002).

Liu, Christian, Ruan et al. (2005) described androgen receptors in the coronary arterial wall, inversely relating their numbers to early atherosclerosis. The absence of these receptors in carotid wall may suggest that androgens receptors have a very limited genomic role in the carotid arterial structure and subsequent development of atherosclerosis. While androgens indubitably play a role in the cardiovascular system, the present study may support the observations that some androgen effects (non-genomic) in arteries are not mediated through androgen receptors (CHOU, SUDHIR, HUTCHINSON et al., 1996; COSTARELLA, STALLONE, RUTECKI et al., 1996; RUBIO, YANEZ, GALLO et al., 1998; RECKELHOFF, ZHANG, SRIVASTAVA et al., 1999; WILLIAMS, LING, DAWOOD et al., 2002). In addition, the present observations suggest that the perceived gender disparity in the structure of the carotid artery with worsening atherosclerosis especially in males may be largely unrelated to the effects of androgens. While there has been controversy as to the role of androgens in the worsening of the carotid intimal medial thickness and subsequent development of atherosclerosis, with some studies showing that androgens are beneficial (HANKE, LENZ, HESS et al., 2001) while others had shown that these hormones are harmful (RAMIREZ, AZCONA, BLASCO et al., 2007), the present study supports neither of the thoughts indicating that androgens may not influence the carotid intimal medial thickness at all.

In conclusion the present data demonstrate that androgen receptors are not expressed in the carotid artery and are not related to carotid IMT, an established marker of atherosclerosis. Androgen receptors may not have a role in the androgenic induced gender differences in carotid intimal medial thickness.

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