

Effects of Orchidectomy and Chronic Androgen Administration on Aortic Media in Male Rabbits

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Abstract: The aim of the present study was to evaluate the effects of orchidectomy and subsequent chronic androgen administration on aortic media in male rabbits. Fifteen adult male rabbits were divided into orchidectomized (Orx), sham-operated controls (SO) and orchidectomized treated with testosterone dipropionate (Orx-TP). In all three groups, the mean area fraction of collagen in the outer half of the vessel was significantly greater than that of the inner half but the reverse is true about elastin ($p < 0.05$). After orchidectomy, the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) decreased significantly in the inner and outer halves and in the whole thickness of the aortic media ($p < 0.05$). Orchidectomy influenced the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) more intensely in the outer than in the inner half of the aortic media. Androgen treatment of orchidectomized rabbits reverses the collagen and smooth muscle cell content of the aortic media to pre-orchidectomy state; however it has little if not effect on reversibility of the medial elastin content.

Key words: Gonadectomy • Aorta • Collagen • Elastin • Smooth muscle cell • Rabbit

INTRODUCTION

Cardiovascular disease (CVD) will remain the major cause of human deaths well into the 21st century [1]. Higher male susceptibility to CVD may be due to genetic, hormonal, or lifestyle factors or a combination of mechanisms [2]. Androgen has been shown to be an important factor contributing to the higher male susceptibility to CVD, through adverse effects on lipids, blood pressure and glucose metabolism [3]. The androgen receptor has been found to be expressed in endothelial cells, smooth muscle cells, macrophages, platelets and cardiomyocytes, all of which are relevant to atherosclerosis and heart failure [2, 4-5]. It has been documented that both male and female sex steroids regulate the extracellular matrix at a number of levels. Testosterone reduces elastin to collagen ratio in rat aorta, whereas estrogen treatment has the opposite effect [6]. Cembrano *et al.* (1960) stated that testosterone has a stimulatory effect on collagen and elastin content of the aorta in both male and female chickens [7]. Seyama *et al.*

(1988) stated that administration of oral contraceptive steroids tended to increase the collagen content, while it did not lower the elastin levels [8].

It is well established that the distributions of elastin and collagen are heterogeneous through the arterial media [9, 10]. However, in the available literature there are no data pertaining to the effect of gonadectomy on scleroprotein or muscle cell content of the different layers of arterial media. The objective of this study, therefore, was to examine the effect of orchidectomy and subsequent androgen administration on scleroprotein and smooth muscle cell content of the outer and inner halves of arterial media in male rabbits.

MATERIALS AND METHODS

Fifteen adult male New Zealand white rabbits (3.3-3.7 kg body weight) bred in our colony were used in the experiment. Animals were maintained on normal rabbit pellets and water *ad libitum*. They were randomly distributed into three groups ($n=5$).

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Two groups, were bilaterally orchidectomized (Orx) under ketamin anesthesia (80 mg/kg). Animals of the control group underwent sham operations (SO). One month after orchidectomy, one Orx group was treated with intramuscular injection of 0.5 ml testosterone propionate (TP). The TP was administrated for eight weeks, at two weeks intervals. Two months later, all the animals were deeply anesthetized with ketamin and fixed by intravascular perfusion via the left ventricle with buffered formalin. Ring segments from the same regions of descending aorta proximal to the first intercostals artery in each animal were removed and immediately fixed by immersion in 10% neutral buffered formalin for 24 h. Paraffin-embedded sections were cut at 5 μ m and stained according to haematoxylin and eosin (H&E) to detect smooth muscle cell, Verhoeff's elastic to detect elastin and Sirius red to detect collagen.

The area fraction of collagen and elastin fibers of the inner and outer halves of the tunica media and the number of smooth muscle nuclei per unit area (mm^{-2}) of the inner and outer halves of the tunica media were measured using the methods previously described by Stergiopoulos *et al.* (2001) [11]. The mean area fraction of collagen and elastic fibers and the mean number of smooth muscle cell nuclei per unit area were compared between the two halves using Student's t test. $P < 0.05$ was considered as significant.

RESULTS

The area fraction of collagen and elastic fibers and the number of smooth muscle cell nuclei per unit area (mm^{-2}) in the outer and inner halves of the aortic media compared in Figs. 1-3. The important findings are as follows:

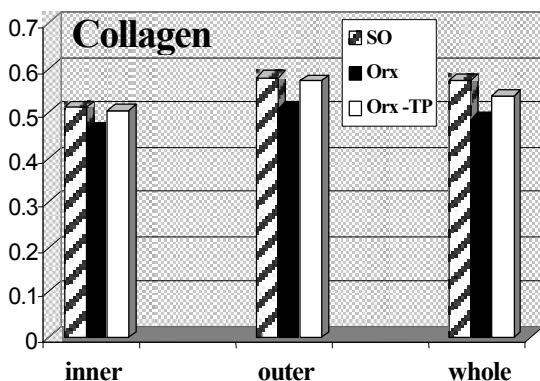


Fig. 1: The mean area fractin of collagen in the inner and outer halves and the whole aortic media in rabbits

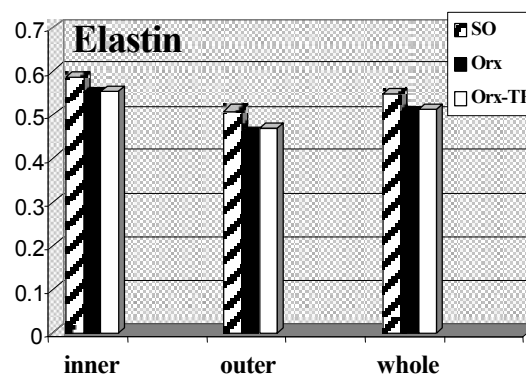


Fig. 2: The mean area fractin of elastin in the inner and outer halves and the whole aortic media in rabbits

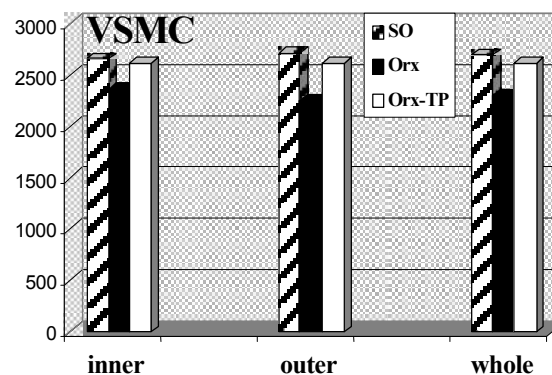


Fig. 3: The mean number of smooth muscle cell nuclei per unit area (mm^{-2}) in the inner and outer halves and the whole aortic media in rabbits

- In all three (SO, Orx and Orx-TP) groups, the mean area fraction of collagen in the outer half of the vessel was significantly greater than that of the inner half but the reverse is true about elastin. The mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) was numerically greater in the outer half of the arterial media than its inner half, but the differences were not statistically significant (Figs. 1-3).
- In the inner half of aortic media of SO rabbits, the mean area fraction of collagen and elastin were 0.512 ± 0.018 and 0.586 ± 0.002 respectively, while the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) was 2667 ± 86 . After orchidectomy, the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) decreased significantly by 9.03% and 6.75% and 11.21% respectively ($p < 0.05$; Figs. 1-3). TP treatment of Orx rabbits reversed these changes. Thus, the mean area fraction of collagen

and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) were 8.98% and 10.86% higher, respectively ($p < 0.05$, Figs. 1, 3) than in the Orx rabbits, so that none of these two morphometric parameters in the Orx-TP rabbits were significantly different from those in the SO rabbits. TP treatment had no statistically significant effect on the mean area fraction of elastin in the Orx animals (Fig. 2).

- In the outer half of aortic media of SO rabbits, the mean area fraction of collagen and elastin were 0.582 ± 0.082 and 0.510 ± 0.066 respectively, while the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) was 2727 ± 62 . Orchidectomy influenced the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) more intensely in the outer than in the inner half of the aortic media. Thus the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) decreased significantly by 12.22% and 9.38% and 17.46% respectively ($p < 0.05$; Figs. 1-3). As in the inner half of the arterial media, TP treatment of Orx rabbits reversed these changes. Thus, the mean area fraction of collagen and the mean number of vascular smooth muscle cell nuclei per unit area were 11.86% and 16.54% higher, respectively ($p < 0.05$, Figs. 1, 3) than in the Orx rabbits, so that none of these two morphometric parameters in the Orx-TP rabbits were significantly different from those in the SO rabbits. However, no statistical significant difference was comparatively verified between the mean area fraction of elastin of the Orx TP and Orx groups (Fig. 2).
- In the whole aortic media of SO rabbits, the mean area fraction of collagen and elastin were 0.574 ± 0.028 and 0.548 ± 0.020 respectively, while the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) was 2697 ± 72 . After orchidectomy, the mean area fraction of collagen and elastin and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) decreased significantly by 14.98%, 7.48% and 14.36% respectively ($p < 0.05$; Figs. 1-3). TP treatment of Orx rabbits reversed these changes. Thus, the mean area fraction of collagen and the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) were 9.46% and 12% higher, respectively ($p < 0.05$, Figs. 1, 3) than in the Orx

rabbits, so that none of these two morphometric parameters in the Orx-TP rabbits were significantly different from those in the SO animals. Again, TP treatment had no statistically significant effect on the mean area fraction of elastin in the Orx animals (Fig. 2).

DISCUSSION

The main structural components of the arterial media are elastin, collagen, vascular smooth muscle cells and ground substance in the form of a mucopolysaccharide gel. The extracellular matrix formed by elastin and collagen fibers is responsible for most of the mechanical strength of arteries [12]. Results obtained from the present study showed that in all three groups, the mean area fraction of collagen in the outer half of the vessel was significantly greater than that of the inner half but the reverse is true about elastin. These findings are consistent with those reported by Feldman and Glagov (1971) in human aortas of young subjects [10]. A similar pattern of an increasing medial collagen/elastin ratio from the intima towards the adventitia has been reported in the bovine aorta [9]. Regarding the area fraction of elastin in outer and inner halves of the arterial media, our finding is inconsistent with that of Stergiopoulos *et al.* (2001) who stated that the area fraction of elastin in the outer half of the aortic media in pig is numerically greater than that of the inner half, although only a small difference of questionable significance was seen between them [11]. Data obtained from the present study also showed that the mean number of vascular smooth muscle cell nuclei per unit area (mm^{-2}) was numerically greater in the outer half of the arterial media than its inner half, but the differences were not statistically significant. This finding is consistent with that of Stergiopoulos *et al.* (2001) who stated that there was no significant difference in the number of vascular smooth muscle cell per unit area (mm^{-2}) when the inner and outer halves of the pig aortic media were compared [11].

Data obtained from the present study also showed that after orchidectomy, the mean area fraction of collagen and elastin decreased significantly in the whole aortic media and in the outer and inner halves of it. This means that androgens may have a stimulatory effect on scleroprotein metabolism in the arterial media. Androgen has been shown to be associated with increases in the concentration of fibrous proteins in gonadectomized chick aortas [7]. Wolinsky (1972) stated that the amounts

of mural fibrous proteins, elastin and collagen, were significantly increased in rats treated with androgen [13]. Cembrano *et al.* (1960) stated that collagen and elastin were significantly higher in males than in females [7]. Gonadectomy in male chickens decreased significantly the content of collagen and elastin, so that the values became similar to those observed in females. They also stated that the treatment of females with testosterone increased significantly the collagen and elastin content of the aorta to levels similar to those observed in males. Fischer *et al.* (1985) stated that female rabbits had elevated collagen deposition when treated with testosterone or progesterone [14].

Our results also showed that after orchidectomy, the mean number of vascular smooth muscle cell per unit area (mm^{-2}) decreased significantly in the whole aortic media and in the outer and inner halves of it. This may be due to the direct stimulatory effect of testosterone on proliferation of vascular smooth muscle cells [15].

Our results also showed that all the examined morphometric parameters in the Orx-TP rabbits were completely reversible except those related to medial elastin content. This may be due to the differences between the turnover rates of elastin and collagen fibers. Mature elastin is extremely stable and its turnover is very slow [16]. Lefevre and Rucker (1980) estimated the turnover and degradation of mature elastin from the aortas of Japanese quail. They concluded that the best estimates of mature elastin turnover are only quantifiable in years [17].

An important unique finding of the present study was the fact that gonadectomy affects the scleroprotein and smooth muscle cell content of the outer half of the arterial media more intensely than its inner half.

As the degree of fibrous protein accumulation may predispose to atherosclerosis [18], it can be stated that the inner half of the arterial media may have more contribution in atherosclerotic changes induced by sex steroid deprivation in old subjects. In the available literature, it has been well documented that vascular remodeling activities induced by mechanical stimuli are not uniform through the arterial media [19]. However, so far, no one has described the different responses of the outer and inner halves of arterial media to sex steroid deprivation.

In conclusion the androgen deprivation in male rabbits influences vascular fibrous protein and vascular smooth muscle cell content of the outer half of the arterial

media more intensely than its inner half. Androgen treatment of gonadectomized rabbits reverses the collagen and smooth muscle cell content of the aortic media to pre-orchidectomy state, however it has little if not effect on reversibility of the medial elastin content.

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