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Research article

Additive damage in the thromboxane related vasoconstriction and bradykinin relaxation of intramural coronary resistance arterioles in a rodent model of andropausal hypertension

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ABSTRACT

Hypertension and andropause both accelerate age–related vascular deterioration. We aimed to evaluate the effects of angiotensin-II induced hypertension and deficiency of testosterone combined regarding the resistance coronaries found intramurally.

Four male groups were formed from the animals: control group (Co, n = 10); the group that underwenr orchidectomy (ORC, n = 13), those that received an infusion of angiotensin-II (AII, n = 10) and a grous that received AII infusion and were also surgically orchidectomized (AII + ORC, n = 8). AII and AII + ORC animals were infused with infusing angiotensin-II (100 ng/min/kg) using osmotic minipumps. Orchidectomy was perfomed in the ORC and the AII + ORC groupsto establish deficiency regarding testosterone. Following four weeks of treatment, pressure-arteriography was performed in vitro, and the tone induced by administration of thromboxane-agonist (U46619) and bradykinin during analysis of the intramural coronaries (well-known to be resistance arterioles) was studied.

U46619-induced vasoconstriction poved to be significantly decreased in the ORC and AII + ORC groups when compared with Co and AII animals. In ORC and AII + ORC groups, the bradykinin-induced relaxation was also significantly reduced to a greater extent compared to Co and AII rats. Following orchidectomy, the vasocontraction and vasodilatation capacity of blood vessels is reduced. The effect of testosterone deficiency on constrictor tone and relaxation remains pronounced even in AII hypertension: testosterone deficiency further narrows adaptation range in the double noxa (AII + ORC) group. Our studies suggest that vascular changes caused by high blood pressure and testosterone deficiency together may significantly increase age-related cardiovascular risk.

1. Introduction

Vascular aging is the process during which the structure and function of blood vessels is damaged over time. These changes together may lead to vascular vulnerability and hypertension [1, 2, 3, 4]. Hypertension related damage seems to be a type of accelerated vascular aging, further increasing cardiovascular risk [5]. Characteristic lesions of vascular damage are endothelial dysfunction, vascular wall remodeling,

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inflammation, athero- and arteriosclerosis and increased vascular wall stiffness [4, 5].

It has long been established that in women menopausal estrogen deficiency significantly increases cardiovascular risk [6, 7]. It is also well known that testosterone deficiency (men with hypogonadism/andropause) also increases cardiovascular risk [8, 9]. Similar to accelerated vascular aging caused by hypertension, andropause may correspond to a specific effect on men that also accelerates vascular aging. The risk of cardiovascular disease (CVD) increases not only directly due to testosterone deficiency [10], but also indirectly, since testosterone deficiency increases the risk of metabolic syndrome, type 2 diabetes, obesity, atherosclerosis, dyslipidemia and hypertension [10]. Contradictory results can be found on the effects of testosterone supplementation in the elderly. On the one hand, testosterone supplementation reduced ischemic symptoms in human angina studies [11]. On the other hand, testosterone supplementation in the elderly (in men over 60 years of age) may have a detrimental effect on the cardiovascular system [12]. Frinke et al. found that in case of men at ages 65 years and older the risk for developing either a stroke or a non-mortal infarction within the myocardium increased during the first 90 days of testosterone treatment, however this risk decreased over time [12, 13]. In another publication, testosterone supplementation increased mortality, and the risk of myocardial infarction and stroke in elderly (over 60 years) men who had a preexisting previous heart condition [12, 14].

Testosterone has a relaxing effect on the coronary arteries: testosterone opens the large-conductance calcium-activated potassium channel. Removal of the endothelium on porcine coronary arteries did not significantly affect testosterone-induced coronary artery relaxation; therefore, it is likely that the vascular smooth muscle cell is the primary site of the vasodilatory effect of testosterone in porcine coronary arteries [15]. Testosterone also relaxes thoracic aorta in rat [16], coronary artery and thoracic aorta in rabbit [17]. Based on previous studies, it is known that testosterone-induced vasorelaxation is independent of the classical androgen receptor [18].

Low testosterone levels alone damage small vessel function: both relaxation and contraction. Testosterone deficiency has detrimental effects on relaxation on one hand through decreased NO production [19] and on the other hand through altered cyclic adenosine monophosphate and potassium channel activation [20]. In contrast, testosterone enhanced thromboxane-induced vasoconstriction in the coronary arteries in male guinea pigs [21]. Testosterone leads to relaxation via nitrogen monoxide (NO) through the production of eNOS in human and in male rats [19, 22]. Myogenic tone also decreases with castration in male rats [23]. The internal pudendal arteries from orchidectomized rats exhibited decreased phenylephrine- and electrical field stimulation induced contraction and decreased acetylcholine- and relaxation values when stimulated by an electric field [24].

Animal studies have found, that as a result of castration, not only increases but also decreases in blood pressure may be observed [20, 25, 26]. To resolve conflicting results, further studies are needed.

However, testosterone deficiency does not only directly damage the function of small blood vessels, but also indirectly. Testosterone deficiency increases the risk of obesity, dyslipidemia, reduction of muscle mass and insulin resistance, which can strongly affect vascular aging including vascular function. Connection of these states with elevated cardiovascular risk is obvious. During a 5-year follow-up examination of adolescents, Ryder et al. found that having type 2 diabetes, being obese and demonstrating elevated values for baseline systolic blood pressure early in life leads to an acceleration regarding the risk of premature vascular aging. Being obese, suffering from type 2 diabetes and having elevated systolic blood pressure values lear to an increase in the thickness of the intima and media of the carotid arteries; it also increased the velocity of the pulse wave from the carotid arteries to the-femoral arteries [27]. In adult Caucasian population without cardiovascular disease, insulin resistance showed a positive association with carotid-to-femoral pulse wave velocity and brachial-to-ankle pulse wave velocity and with early vascular ageing (the individuals with values of carotid-to-femoral pulse wave velocity or brachial-to-ankle pulse wave velocity over 90th percenteliles) [28, 29]. The investigated values characterizing lipid profile (low-density lipprotein levels, triglyceride non-high-density, lipoprotein and also total cholesterol levels) strongly correlated with increased pulse wave velocity values and early vascular aging. Furthermore, triglyceride/high-density lipoprotein ratio might be used for prediction to early vascular aging [30]. There is a reciprocal relationship between sarcopenia and vascular aging: advanced vascular aging leads to a decrease in skeletal muscle mass, while sarcopenia (loss of muscle mass and strength) increases cardiovascular burden [31].

Like testosterone, high blood pressure also affects the function of small blood vessels. Hypertension also increases myogenic tone and thromboxane—induced contraction [32, 33], while endothelium -dependent dilatation deteriorates [34]. As an initial step in hypertension in coronary resistance vessels, wall tension and elastic modulus increase in male rats, while inward eutrophic remodeling may be observed in female animals. Vessel tone and contractile responses to thromboxane agonist increase in both sexes, but this is significantly more pronounced in males, while decrease in vasorelaxation is bigger in females [35].

There is significantly less data available regarding effects of a deficiency of testosterone combined with hypertension on resistence arteries [26, 36]. The effects of testosterone-deficiency and hypertension together on the morphology and also on the biomechanical characteristics of resistance coronaries were first examined by our team [37]. In brief: as a result of hypertension, spontaneous tone increases first, even without remodeling of wall structure. Testosterone deficiency alone results in inward hypotrophic remodeling, which persists in double noxa. Hypertensive stimulus with testosterone deficiency further reduces vessel diameter [37]. However, little is known about the functional adaptation of blood vessels affected by the double noxa [26].

In the present study, the effects of a surgically established deficiency of testosterone together with hypertension was investigated on the coronary resistance arterioles.

2. Materials and methods

2.1. Chemicals

Rats were anesthetized with Euthasol (by CEVA Santé Animale, Liboume, France). Hypertension was established via an Angiotensin (AII) modelas described previously [32, 37, 38, 39]. An osmotic minipump (by Alzet, 2ML4, Durect Co, Cupertino, US) containing AII (Sigma-Aldrich Co, St. Louis, Missouri, US and Budapest, Hungary) was implanted subcutaneously. The experiments were conducted under in vitro conditions; physiological Krebs-Ringer (nKR) solution was used. The nKR solution was composed as follows (in mmol/l) 119 NaCl, 1.2 NaH2PO4, 4.7 KCl, 1.17 MgSO4, 2.5 CaCl2, 24 NaHCO3,5.5 glucose and 0.0345 EDTA (Reneal, Budapest, Hungary). The calcium-free Krebs solution - used to achieve total relaxation within the vascular smooth muscle tissue – was composed of the following (in mmol/l): 4.7 KCl, 1.18 NaH2PO4, 92 NaCl, 20 1.17 MgSO4, MgCl2, 24 NaHCO3, 2 EGTA 5.5 glucose, and 0.025 EDTA (Reneal, Budapest, Hungary). U46619 (a TxA2 receptor agonist) and bradykinin-acetate (BK). All chemicals had a purity greater than 98% (Sigma-Aldrich, St Louis, Missouri, US and Budapest, Hungary) and they were all prepared in the nKR solution on the day of the experiment.

2.2. Animals and animal care

Throughout the experiments, the relevant regulations and guideline of the Institute for Laboratory Animal Researches and the National Society for Medical Research (published in the National Institutes of Health Publication, No. 86–23, revised 1996) were adhered to when using and caring for the animals during the experiment series. The study was accredited at Semmelweis University (by the dedicated Animal Care

Committee) and also by the relevant Hungarian authorities (PEI/001/820–2/2015 and PE/EA/1427–7//2018).

Young adult Sprague-Dawley male rats (n = 41, 2-month-old, 280–320 g) were purchased from Innovo Kft (Gödöllő, Hungary); they were housed at room temperature (22 ± 2 °C), and were provided a light-dark cycles of 12 h. Standard rat chow and tap water were available to the animals ad libitum.

After seven days acclimatization, they were divided into the following four groups: control (Co, n = 10); those undergoing surgical orchidectomy (ORC, n = 13), those receiving an infusion of AII (AII, n = 10) and the AII infused and surgically orchidectomized group (AII + ORC, n = 8). The animals that received a treatment of AII (AII and AII + ORC) had osmotic minipumps surgically implanted under anesthesia (45 mg/kg intraperitoneal pentobarbital), subcutaneously into the region above the lumbar spine. The infusion rate of the AII infusion minipump was 100 ng/kg/min, which leads to chronic blood pressure elevation without any acute pressure effects in 2–3 weeks [37, 38, 39]. We choose this AII hypertension model to study coronary arteries alterations in the early hypertensive state. 100 ng/mg/kg/min is a sub-pressor dose; therefore, it does not have acute hypertensive effects. Administered chronically, it leads to the development of hypertension. We choose week 4 in our series to perform experiments as this corresponds with the early stages of hypertension. In our previous publication [37] this AII infusion increased both systolic and mean blood pressure values in compared to the Co rats. Hormone deficiency lead to decreases blood pressure in the group of hypertensive rats (AII + ORC) compared with the rats that did not undergo orchidectomy AII-infused animals. Mean arterial pressure values from the study groups were found to be the following: Co: 114 ± 6 mmHg; ORC: 102 ± 7 mmHg; AII: 134 ± 7 mmHg and AII + ORC: 114 ± 8 mmHg [37].

Orchidectomy is as an established andropause model [37, 40, 41]. In this study it resulted in the following androgen levels: 2.2 ng/ml in intact and 0.1 ng/ml in orchidectomized rats [40, 41]. To perform orchidectomy, animals (ORC and AII + ORC) under anesthesia (45 mg/kg, intraperitoneal), the testis was removed surgically at the posterior tip of each scrotum as described previously [37]. In the AII + ORC group, the removal of testis and osmotic minipump implantation were performed at the same time. Neither medical nor surgical complications occurred during the course of treatment.

2.3. Pressure arteriography of coronary arterioles

AT the end of the experimental series body weight was measured, values were as follows: Co, 393 ± 9 g; ORC, 396 ± 5 g; AII, 416 ± 9 g and AII + ORC, 401 ± 13 g (non significant with two-way ANOVA) [37]. The animals were then anaesthetized as before, the heart removed through the chest and intramural resistance coronary branches with similar in situ outer diameters (ca. 200 μm) were carefully dissected and isolated under a stereomicroscope from secondary branches of the left anterior descending coronary [42]. These arteriolar segments with a length of approximately 2 mm were removed, placed in a vessel chamber filled with nKR, and cannulated at both ends using plastic microcannulas. Finally, they were extended to their normal, in situ, in vivo length. The temperature of the nKR was set at 37 °C and it was also bubbled with predetermined ratio of gasese (20% O_2, 75% N_2 and 5 % CO_2, – this stabilized pH at 7.4). Theisolated cannulated vessel segments were mounted and pressurized on the pressure-servo-systems (Living Systems, Burlington, VT, US) under no-flow conditions.

The arterioles' inner diameter was measured after acquiring microscopic images of the vessels (aka "microangiometry"). This experimental setup contained a glass-bottom tissue bath positioned under an inverted microscope (Leica), centered right into a path of light to visualize alterations of the inner diameter of the arteriole segment. A DCM 130 E camera captured digital images of the isolated segments. Processing and analysis of the acquired microscopic images was performed offline by dedicated image-analyzing software (Scope Photo). A micrometer etalon was used to calibrate length (Wild, Heerbrugg, Switzerland).

Contractile characteristics of the studied isolated vessel segments was performed as follows: equilibration for 30 min at 50 mmHg to allow for establishment of myogenic tone [43]. This step was necessary to check the viability of the vessels. Following equilibration, the steady-state vessel diameter was photographed. Pressure-diameter curves were recorded following two consequent conditioning pressure cycles (2-90-2-90-2 mmHg). Then pressure was increased to 30 mmHg and then to 50, 70 and 90 mmHg. Inner diameter values were measured at pressure value following equilibration. TxA2 agonist (U46619-concentration of 10^{-6} M) was added to the tissue bath and incubation was allowed for 10 min at 50 mmHg; at this point an image of the steady-state diameter was captured. Pressure-diameter curves were then recorded sequentially and repeatedly. Inner diameter values were always measured at each pressure value. Without washing out the U46619, bradykinin (BK) was added in 10^{-6} M concentration, and a further 20 min of incubation at 50 mmHg was allowed before capturing the image of the steady–state diameter. Thereafter, the pressure diameter curves were recorded repeatedly. The inner diameter was measured at each step. Finally, all drugs were washed out with calcium-free Krebs-Ringer solution, and after a final 30 min of incubation at 50 mmHg, an image of the relaxed vessel diameter was taken and the experiments were finished by taking the pressure diameter curves with the fully relaxed muscle (passive state). Inner diameter was measured at each step.

2.4. Contractility calculations

The following characteristic parameters were calculated based on the data from the pressure-diameter curves:

- Mogenic tone (%)

$$T_{nKR} = (r_{iCa\text{-}free} - r_{inKR})/r_{iCa\text{-}free} * 100$$

- U46619-induced constriction (%):

$$C_{U46619} = (r_{inKR} - r_{iU46619})/r_{iCa\text{-}free} * 100$$

- Bradykinin-induced relaxation (%)

$$R_{BK} = (r_{iBK} - r_{iU46619})/r_{iCa\text{-}free} * 100$$

where $r_{iCa\text{-}free}$ and r_{inKR} are values representing inner radii measurements taken in calcium-free and in a nKRr at the same pressure. $R_{iU46619}$ and r_{iBK} are measurement values of inner radii following application of TxA2 agonist (U46619) and bradykinin - at the same pressure points, respectively. Inner radius data can be found in the supplementary data.

2.5. Statistical evaluation

Statistical comparison of the measured data was performed by SPSS Sigma Stat and GraphPad Prism 6.0 softwares. We have presented all of our data mean ± SEM. Shapiro-Wilk method was used to assess normal distribution. In case of repeated measures data (pressure curves) mixed-effects models was performed. We applied Tukey's post hoc in the mixed-effect models. Statistical significance was considered at $P < 0.05$. GraphPad Prism 6.0 software was used to plot Figures. P values and Tukey's post hoc test numbers are found in the supplementary material.

3. Results

3.1. Contractility parameters of intramural coronary resistance arterioles

Vascular contractility was checked by myogenic tone derived from the inner radius of the coronary vessels. In the AII group, myogenic tone was significantly higher compared to both the Co and ORC groups.

Orchidectomy alone did not alter myogenic tone. However, due to double noxa (in the AII + ORC group), the myogenic tone was significantly higher compared to the ORC group (Figure 1).

Thromboxane induced (U46619) vasoconstriction did not differ between Co and AII groups, but was significantly reduced due to castration in both the ORC and AII + ORC groups compared to Co animals. The AII + ORC group differed significantly different not only from the group of controls (Co) but also from the AII group. Compared to the ORC group, no further reduction in thromboxane-induced vasoconstriction was observed with the combined effect of AII treatment and testosterone deficiency (in the AII + ORC group) (Figure 2).

Endothelial dilatation was tested with bradykinin, which did not change to AII treatment alone compared to the control group (Co vs. AII group). Due to testosterone – deficiency, a decrease in relaxation was observed compared to the Co group and AII groups (Co vs. ORC and AII vs. ORC). Combined noxa of hypertension and testosterone–deficiency decreased bradykinin induced relaxation compared to the Co group and the AII only group (Co vs. AII + ORC group and AII vs. AII + ORC group), but there was no difference compared to orchidectomy alone group (ORC vs. AII + ORC) (Figure 3).

4. Discussion

In our current study, we investigated the effects of a surgically established deficiency of testosterone together with hypertension on the characteristics of intramural coronaries of male rats. The major findings of our investigation may be summarized as follows [1]: Testosterone–deficiency alone impaired vascular reactivity; decreased both thromboxane induced contraction and bradykinin dependent relaxation [2]; the effect of testosterone deficiency on constrictor tone and relaxation can be detected both in AII hypertension and in normotensive animals [3]; double noxa, that is AII hypertension and testosterone deficiency together, resulted in a combination of abnormalities observed in both the AII group and ORC group: myogenic tone increased, capacity for both contraction and relaxation decreased, further narrowing the range of vascular adaptation relative to ORC and AII groups, however, the association of noxa does not alter what was altered by the one factor. The results from our study may well contribute to the better understanding of the initial steps of age-related impairment found regarding

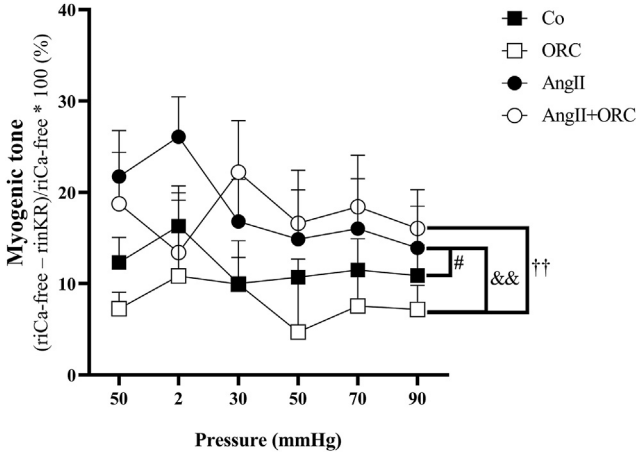

Figure 1. Myogenic tone is plotted on this figure as a function of intraluminal pressure. The inner radii values of the intramural coronaries from the Co, ORC, AII and AII + ORC groups were was measured under passive conditions (in Calcium-free solution) of. Myogenic tone found regarding coronaries in both the AII and in the AII + ORC groups was significantly higher compared to Co and ORC groups. Data in this figure are expressed ase mean (+/-SEM) values. We used mixed-effects analysis at the same pressure. The significant values from Tukey's post hoc tests regarding the 4 investigated groups are shown. #P < 0.05 Co vs. AII; && P < 0.01 ORC vs. AII; ††P < 0.01 ORC vs. AII + ORC.

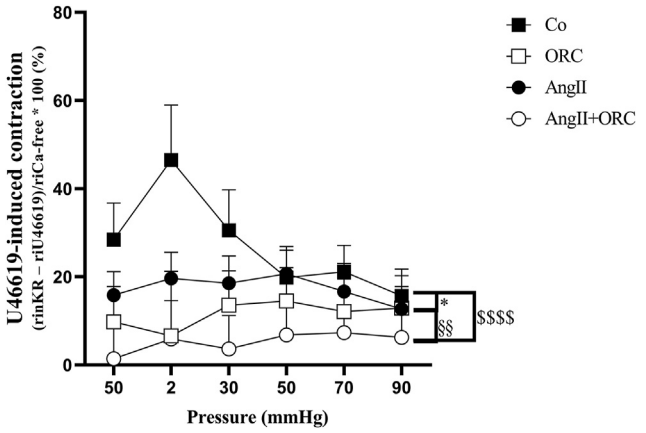

Figure 2. U46619-induced contraction is plotted in this figure as a function of the intraluminal pressure values measured in the intramural coronary arteries from the Co, ORC, AII and AII + ORC animals. Vasoconstriction induced by U46619 was significantly less in the coronaries of the orchidectomized animals (ORC and AII + ORC groups) compared to that Co and AII rats. We expressed data as mean (SEM) values. We used mixed-effects analysis at the same pressure. . The significant values from Tukey's post hoc tests regarding the 4 investigated groups are shown. *P < 0.05 Co vs. ORC; $$$$ P < 0.0001 Co vs. AII + ORC; §§P < 0.01 AII vs. AII + ORC.

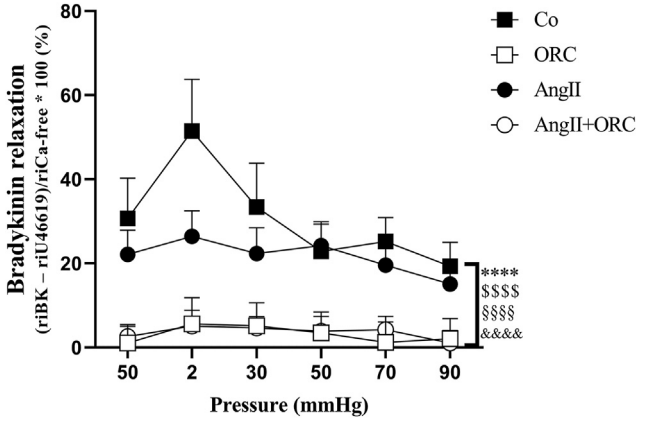

Figure 3. Bradykinin relaxation is plotted in this figure as a function of intraluminal pressure measured of the intramural coronary arteries from the Co, ORC, AII and AII + ORC animals. Relaxation induced by Bradykinin was significantly decreased in the group that underwent orchidectomy (ORC and AII + ORC groups) compared to values from the Co and AII animals. We expressed data as mean (SEM) values. We used mixed-effects analysis at the same pressure. The significant values from Tukey's post hoc tests regarding the 4 investigated groups are shown. ****P < 0.0001 Co vs. ORC; $$$$ P < 0.0001 Co vs. AII + ORC; §§§§P < 0.0001 AII vs. AII + ORC and &&&& P < 0.0001 ORC vs. AII.

vascular function in hypertension and in andropause in males, these result in an increase of cardiovascular morbidity and mortality.

Our animal model provides an opportunity to study early vascular damage caused by hypertension and testosterone–deficiency together. It should be emphasized from our previous results in female animals, that our early hypertension model is suitable for the detection of initial lesions. This is the first study where the effects of both hypertension and testosterone hormone depletion were studied on the functional characteristics of intramural coronaries (contractile and vasodilatative capacity) via a sub-pressor dose angiotensin II-induced hypertension model. The etiopathogenesis of vascular aging is heterogeneous, it is influenced by many factors. Angiotensin II stimulates the formation of superoxide anion $O2^-$ through the activation of membrane-bound NAD(P)H-oxidase with the type 1 angiotensin receptor, which contributes to the reduction

of NO bioavailability, vascular damage and atherogenesis [44, 45]. Furthermore, in diabetic men, decreased levels of testosterone were linked to both elevated total and mitochondrial reactive oxygen species as well as to the decreased function of superoxide dismutase and glutathione S-transferase (expression of both was decreased): testosterone levels and ROS production were found to correlate negatively [46]. Inflammatory cytokines, specifically interleukins (IL-2, IL-6, IL-10, IL-12 and IL-13) were increased when testosterone levels decresed, further, testosterone supplementation decreased interleukin levels [47]. In our model the vascular damaging effects could be studied both independently for hypertension and for lowered testosterone levels in males as well as for their combined action.

In our present study, testosterone-deficiency decreased both contractility and endothelial relaxation in the ORC group. Data in the literature are contradictory regarding the effects of testosterone deficiency and supplementation on the cardiovascular system. One of the acute extra-nuclear effects of testosterone is that it increases the capacity for arterial dilation in the mesenteric arteries, aortic rings and in the basilar arteries of different species [48]. In physiological concentrations these effects are endothelium-dependent, however, supra-physiological doses lead to endothelium-independent dilatory action [48]. Acute doses of testosterone resulted in endothelium-independent vasorelaxation in coronaries and the aorta both in males and females [17]. However, it is not the acute vasodilator effect of testosterone that we observe following castration or castration + testosterone supplementation. Using the Langendorff model it was observed in the coronaries of male rats, that BK induced vasodilation is impaired following castration, and this was restored by supplementation of physiological and supra-physiological doses of testosterone [48]. In contrast, in human studies it was observed, that physiological supplementation of testosterone weakened vascular reactivity in those with androgen deficiency [49]. In an other study castration decreased electrical field stimulation (EFS) induced contraction in mesentery vessels and increased vasodilator response [50]. Changes in contraction on different types of vessels (mesenteric arteries and aorta) following castration have also been described, but site-specific action in coronary arterioles could not be excluded. As a result of castration the quantity of TxA2 produced by smooth muscle cells increases in mesenteric arteries [51]. TxA2 release also increases in the aorta and in mesentery arteries shortly following castration [52]. Furthermore, as a result of orchidectomy, the phenylephrine- and EFS induced contraction and acetylcholine- and EFS induced relaxation decreases in the internal pudendal arteries from rats [24]. In our current study, castration alone did not decrease myogenic tone, however U46619 induced tone were decreased following castration compared to values found in the control group. Bradykinin-induced vasodilation was decreased as well.

An early feature in hypertensive lesions is an increase in myogenic tone [53]. In our current study, similar to our previous publications [37], myogenic tone was significantly elevated in the AII group.

Our studies demonstrated that andropause-induced reduced contraction and relaxation ability appear not only in normotension, however under the conditions of drug-infusion hypertension. Reduced vascular adaptation ability of testosterone plus hypertensive animals significantly differed from those subjected to hypertension alone. Furthermore, in the double noxa group, we found not only the adverse effects caused by testosterone deficiency but also myogenic tone increase caused by AII treatment, so all three differences were observed in the double noxa group (myogenic tone increase, decreased contractility and relaxation ability). The combined presence of the two damaging factors did not alter what was altered by the single factor. The effects of hypertension and castration have been studied previously on mesenteric arteries and on extremity arteries as well [26, 36, 54, 55]. However, data are contradictory. In spontaneously hypertensive male rats, autonomic venous tone was found to be significantly reduced by castration [36]. In mesenteric arteries of spontaneously hypertensive male rats, double noxa, in parallel with our results, weakened the serotonin-induced vasoconstriction, but slightly worsened endothelial dilatation [26]. In Ang II-induced hypertension, contractility increased in mesenteric arteries. However, this was prevented by castration and restored by supplementation of testosterone [55]. These conflicting results may be due to different types of vessels (coronary vs. mesenteric artery, aorta, musculocutaneous arteries), or different types of animals (Sprague Dawley rats vs. spontaneously hypertensive rats).

We know from previous studies, that both bodybuilders and athletes, using anabolic steroids and men with andropause are more at risk for cardiovascular morbidity and mortality than fertile-age men with physiological hormone levels [9, 56]. Based on this, our present study might describe one of the potential mechanisms underlying the cardiovascular vulnerability of andropausal men.

In addition, based on our results, there is rationale for hormone replacement to physiological levels in andropausal men to reduce the vulnerability of the vascular system. Such protective effect can be expected both in normotensive and hypertensive conditions. We suggest to expect the cardiovascular effects of testosterone in form of an “U” shaped curve: both too high and too low testosterone levels can be expected to deviate from the optimum, emphasis should be put to ensure optimal testosterone levels. Therefore, in case of androgen deficiency supplementation of testosterone to an optimal level may have a protective effect in terms of cardiovascular disease [57, 58, 59]. Of course, this protective effect is assumed to start at the onset of hormone deficiency. In an analogue situation of women the positive effects of menopausal hormone replacement are noticeable only if hormone replacement is started within 5 years of the onset of menopause [6]. Beyond 10 years, definitive vascular damage develops, and complications outweigh the benefits of treatment [60]. Similar advantages and disadvantage may also be assumed regarding males in the andropause. Our line of reasoning should be supported by further animal and clinical studies, but contrary to the paradigm, our results support that restoring physiological hormone levels in andropause may reduce cardiovascular risk.

The limitation of our study is the lack of histology or immunoblotting examinations. Direct study of these vessels cannot be studied ethically in humans; therefore animal testing is of paramount importance. Although human conditions cannot be inferred directly from our animal experimental result, they may provide perspective for the design of clinical trials.

5. Conclusion

Based on our present results, both noxa resulted in different and unfavourable changes in vessel function. That is, multiplicative, noxa-specific and consistently developing changes –independent from each other - resembling accelerated vascular aging have been detected in this early model with slight differences. After all, there was an increase in myogenic tone in both AII groups and a decrease in TXA and BK tones in both ORC groups, and all of these were observed together in the double noxa group, however, the association of two harmful noxa does not alter what was altered by the single factor. These are the initial changes that later can be expected to induce definitive target organ damage. Our results call attention to the fact, that similar to female menopause, andropause also increases cardiovascular vulnerability. More importantly, our results were obtained from resistance coronaries - responsible for the blood supply of the heart in a direct manner. The condition of these vessels may also play a key role in heart function and may determine cardiovascular ischemic events.

Declarations

Author contribution statement

Attila Jósvai; Judit Hetthéssy: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marianna Török: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Máté Mátrai; Anna Monori-Kiss; Jennifer Makk; Márton Vezér; Levente Sára; István Szabó: Performed the experiments; Analyzed and interpreted the data.

Béla Szakács; György L. Nádasy; Szabolcs Várbíró: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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