COMBINED EFFECTS OF HYPERTENSION AND TESTOSTERONE DEFICIENCY ON CORONARY RESISTANCE ARTERIES

Ph.D. thesis

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1. Introduction

As female menopause seems to increase cardiovascular risk, similar observations can be made in men - andropause has also an adverse effect. Testosterone deficiency may be associated with risk factors for cardiovascular diseases, such as high level of low-density lipoprotein, high blood sugar level or high levels of pro-inflammatory cytokines. Hypertension alone greatly increases cardiovascular risk by damaging small and large blood vessels and by overloading the heart.

Therefore, in our current research we investigated the possible mechanisms through which testosterone deficiency and hypertension damage intramural coronary vessels – which ones are directly responsible for myocardial blood supply.

2. Objectives

Both andropause and hypertension has detrimental effect on vascular function. Furthermore, they separately also increase the risk of coronary artery damage. Biomechanical properties of the coronary arteries or arterioles have only been studied more narrowly in this regard so far. More specifically, the dual effects of male hormone -depleted state and chronic angiotensin II administration on the function of coronary resistance arterioles has not yet been analyzed or been proposed to influence cardiovascular vulnerability. Therefore, in our present study, we focused on the morphological, biomechanical, and functional changes of coronary arterioles when both noxa are present.

Based on this background, we aimed at investigating:

1) the morphological and biomechanical properties of intramural coronary arterioles when testosterone – deficiency and hypertension are both present together and whether there is a connection between the damage caused by these two noxa with regard to the biomechanics of intramural coronary vessels.
2) the contractility of intramural coronary arterioles under similar circumstances, and the interactions between hypertension and testosterone deficiency in vascular function and contractility.

3. Methods

3.1. Animals

Throughout the experiments, all animals housed and kept according to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). All procedures and handling of the animals during the study were approved by the Animal Care Committee of Semmelweis University as well as by the Hungarian state authorities (permission number: PEI/001/820-2/2015). A total of 41 sexually mature, 2-month-old male Sprague-Dawley rats were housed under standard laboratory conditions, with 12 hour light-dark cycles. The rats were distributed into four groups, control male (Co, n=10); orchidectomized male (OCT, n=13), angiotensin induced male (AII, n=10) and AII induced plus orchidectomized male (AII + OCT, n=8). The angiotensin treated groups (AII and AII +

OCT) were subjected to subcutaneous implantation of osmotic minipump performed under anesthesia and sterile conditions. Subcutaneous AII infusion rate of the osmotic pump was 100 ng/bwkg/min. Previous studies reported that this is a sub-pressor dose, which led to chronic blood pressure elevation after 2 to 3 weeks without acut blood pressure elevation, which is why we chose this model to study early hypertensive vessel alterations (MS1 20, 21). In the OCT and AII + OCT group, orchidectomy was performed using standard surgical techniques under pentobarbital anaesthesia. In the AII + OCT group, orchidectomy and osmotic pump implantation were performed in a single session.

3.2. Pressure arteriography of coronary arterioles

Following 4 weeks of AII treatment, under pentobarbital anesthesia (45 mg/kg body weight, given intraperitoneally), the blood pressure was measured via right carotid artery cannulation, the heart was removed, its weight measured. The intramural coronary arterioles, secondary branches of the left anterior descending coronary artery (with an in situ outer diameter of around 200 μm), were isolated under high magnification using careful microtechniques. These arteriolar segments with a length of approximately 2 mm were removed, placed into a vessel chamber filled with nKR, and microcannulated at both ends with plastic microcannulas. Finally, they were extended to their normal, in situ, in vivo length. The constant temperature of the bath was 37 °C and it was bubbled with a gas mixture of 5 % CO_2, 20% O2 and 75% N2, which stabilized its pH at 7.4. The temperature of the nKR was set to 37 °C. The composition of the gas mixture, with the

nKR was bubbled was 5 % CO2, 20% O2 and 75% N2, which stabilized its pH at 7.4. The plastic microcannulas were connected to servo-controlled roller pumps (Living Systems, Burlington, VT, US) to set the intraluminal pressure, and the arterioles were pressurized under no-flow conditions. The inner - outer diameters and wall thickness of the arterioles were measured by microangiometry. In this setup, the glass-bottomed tissue bath was positioned on the stage of an inverted microscope (Leica) to visualize the diameter changes of the arteriole. Magnified pictures of the vessels were acquired by a DCM 130 E camera. Subsequent offline analysis and processing of the acquired microscopic images was carried out with the aid of a specific image-analyzing software (ScopePhoto). Length calibration was performed using a micrometer etalon (Wild, Heerbrugg, Switzerland).

3.3. Vascular protocols

The coronary arterioles were allowed to equilibrate for 30 minutes at 50 mmHg, in a normal Krebs solution, the steady-state vessel diameter was measured. Then, pressure-diameter curves were obtained after two conditioning pressure cycles (2-90-2-90-2 mmHg). The pressure was increased first to 30 mmHg and then up to 50, 70 and 90 mmHg. The inner diameter was measured at each step. As a next step, a TxA2 agonist (U46619, at a concentration of 10^{-6} M) was added to the nKR, and following 10 minutes of further incubation at 50 mmHg, the steady-state diameter was photographed again. Thereafter, the pressure diameter curves were recorded repeatedly. Without washing out U46619, bradykinin (BK) was added in 10^{-6} M concentration, and a further 20 minutes of incubation at 50 mmHg was allowed before capturing the image of the steady-state diameter. Thereafter, the pressure diameter curves were repeatedly recorded. Finally, all drugs

were washed out with calcium-free Krebs-Ringer solution, after 30 minute incubation at 50 mmHg, the experiments were completed by taking the pressure-diameter curves. Inner, outer diameter and wall thickness were measured at each step.

3.4. Biomechanical calculations

The biomechanical parameters from the calcium-free Krebs solution data were calculated as follows:

- Wall thickness (h) = r_o-r_i
- Wall thickness to lumen diameter (Q): $Q=h/d_i$
- Cross sectional area (A_w): $A_w=(r_o^2-r_i^2)*\pi$
- Tangential wall stress (σ): $\sigma=(P*r_i)/h)$, according to the Laplace-Frank equation
- Incremental tangential elastic modulus of the cylindrical segments (E_{inc}): $E_{inc}=(2r_o ri^2*\Delta P)/((r_o^2–r_i^2)*\Delta r_o)$

where h is the wall thickness, r_o and r_i are the actual values of the outer and inner radii, d_i is the inner diameter, P is the transmural (intraluminal) pressure, and Δr_o is the alteration of the outer radius during a pressure rise of ΔP, according to Cox.

3.5. Contractility calculations

From the pressure-diameter data, the following parameters were calculated:

- Spontaneous tone: $T_{nKR}=(r_{iCa\text{-}free}–r_{inKR})/r_{iCa\text{-}free}*100$ (%)
- U46619induced constriction: $C_{U46619}=(r_{inKR}\text{-}r_{iU46619})/r_{iCa\text{-}free}*100$(%)
- Bradykinin-induced relaxation: $R_{BK}=(r_{iBK}\text{-}r_{iU46619})/r_{iCa\text{-}free}*100$(%)

where $r_{iCa\text{-}free}$ and r_{inKR} are the inner radii measured in a calcium-free solution and in a normal Krebs-Ringer solution at 50 mmHg. $r_{iU46619}$ and r_{iBK} are the inner radii measured after

TxA2 agonist (U46619) and bradykinin at 50 mmHg, respectively.

3.6. Statistical evaluation

For statistical analysis, data measurements were compared by SPSS Sigma Stat software. Data are presented as the mean ± SEM. Normal distribution was tested with the Shapiro-Wilks method. In case of normal distribution, two-way analysis of variance (ANOVA) with the factors 'hypertension' and 'castration' was performed. If there were interactions between 'hypertension' and 'castration' ($p_{int} < 0.05$) in the two-way ANOVA, we used one-way ANOVA.

In case of spontaneous tone, U46619 induced constriction and bradykinin-induced relaxation mixed-effect models was permormed. As a post hoc test, Tukey's post hoc test was used in one-way ANOVA, two-way ANOVA and in mixed-effect models. In case of non-normal distribution, Kruskall-Wallis test with Dunn's post hoc test was performed. A *P* value of < 0.05 was considered as the criterion for statistical significance.

4. Results

4.1. Physiological parameters

After four weeks of AII treatment the heart weight increased significantly in the AII group compared to the control rats. Systolic blood pressure and arterial mean pressure was significantly higher in AII rats than in control animals. The diastolic blood pressure was significantly lower in the AII + OCT groups compared to the AII animals (Table 1.).

Table 1. Basic characteristic and blood pressure parameters of the study groups.				
Variable	Co	OCT	AII	AII + OCT
Basic characteristic				
BW (g)	393 ± 9	396 ± 5	416 ± 9	401 ± 13
HW (g)	1.120 ± 0,03	1.195 ± 0.03	1.29 ± 0.02#	1.22 ± 0.08
HW/BW (g/kg)	2.85 ± 0.06	3.02 ± 0.07	3.08 ± 0.07	3.09 ± 0.13
Blood pressure data				
Systolic blood pressure	122 ± 6	118 ± 7	149 ± 7#	136 ±9
Diastolic blood pressure	109 ± 6	94 ± 7	126 ± 7	102 ± 7§
Mean arterial pressure	114 ± 6	102 ± 7	134 ± 7#	114 ± 8
BW, body weight; HW, heart weight. TWO-WAY ANOVA (factors: hypertension, orchidectomy) with post hoc Tukey's test. Values are the means ± SEM. #P <0.05 Co. vs. AII; §P < 0.05 AII vs. AII + OCT.				

4.2. Morphology parameters of intramural coronary resistance arteries

Even though all harvested l segments were identical in regards to both morphology and anatomy during dissection, significant differences were found regarding the inner radius of the vessels (Fig. 1). Measurements regarding inner radius of the vessels was reduced in the AII + OCT rats compared to all the other animals (control, the AII and OCT). (Fig. 1). Wall thickness was rmeasured to be markedly reduced in the OCT rats compared to the control animals (Fig. 2 Wall thickness to lumen ratio was significantly higher in the AII + OCT group, compared to AII group, and also the OCT anilams (Fig.3). In

addition, as an effect of orchidectomy, the wall cross-sectional area was decreased compared to the control and AII groups (Fig. 4), indicating the development of inward hypotrophic remodeling in the OCT and AII + OCT groups.

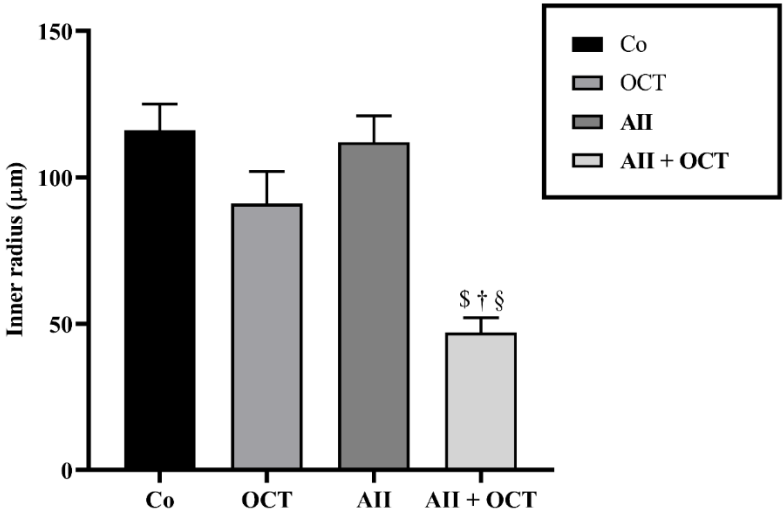

Figure 1. Inner radius in the passive condition

ONE-WAY ANOVA with post hoc Tukey's test. All values are expressed in mean ± SEM. $P < 0.05 Co vs. AII + OCT; †P < 0.05 OCT vs. AII + OCT; §P < 0.05 AII vs. AII + OCT

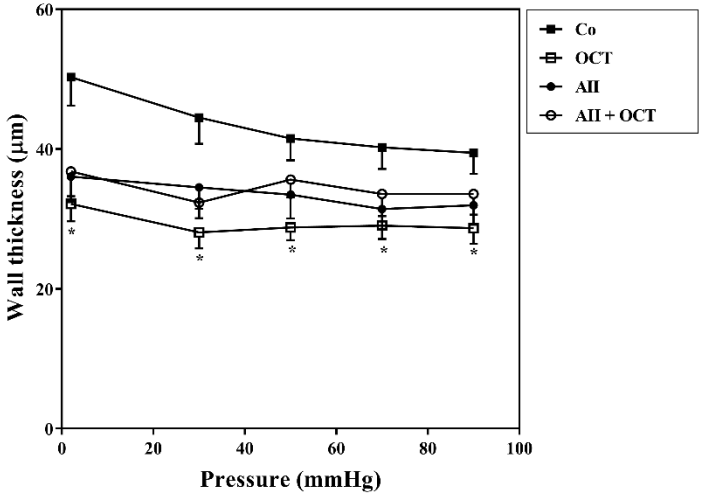

Figure 2. Wall thickness in the passive condition

ONE-WAY ANOVA at same pressure with post hoc Tukey's test. All values are expressed in mean ± SEM. *P < 0.05 Co vs. OCT.

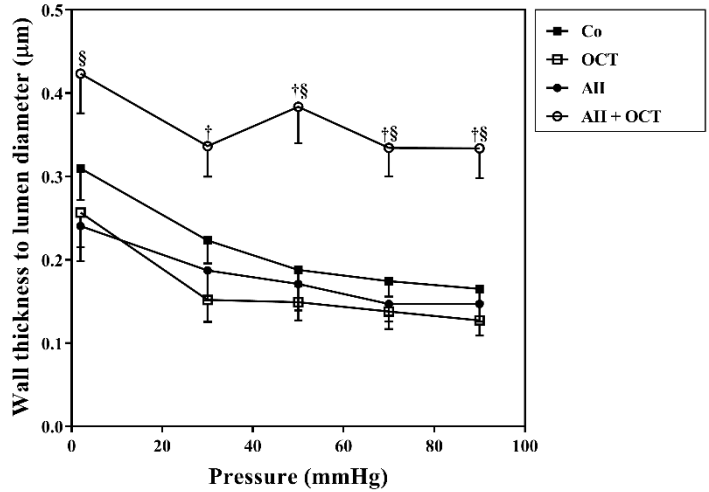

Figure 3. Wall thickness to lumen diameter in the passive condition

KRUSKAL-WALLIS test at the same pressure with post hoc Dunn's test. All values are expressed in mean ± SEM. †P < 0.05 OCT vs. AII + OCT; §P < 0.05 AII vs. AII + OCT.

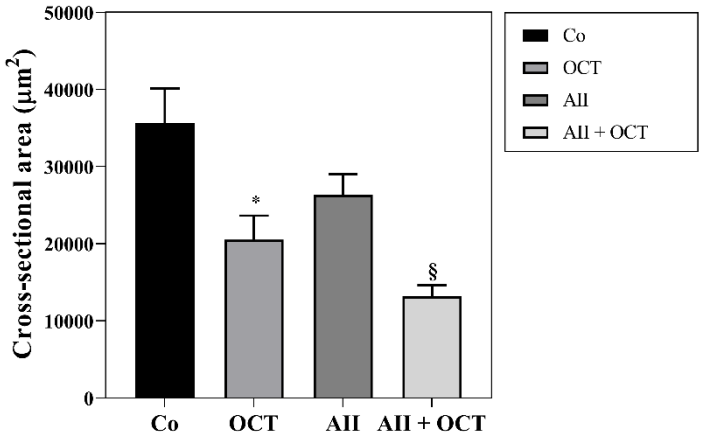

Figure 4. Wall cross-sectional area in the passive condition

TWO-WAY ANOVA (factor: hypertension, orchidectomy) with post hoc Tukey's test. All values are expressed in mean ± SEM. *P < 0.05 Co vs. OCT; §P < 0.05 AII vs. AII + OCT.

4.3. Biomechanical parameters of intramural coronary resistance arteries

The mechanical loading of the coronary artery wall, the tangential wall stress was significantly lower in AII + OCT animals than in AII animals, and also than OCT rats (Fig. 5). There was no significant difference among the incremental elastic moduli of the vessels.

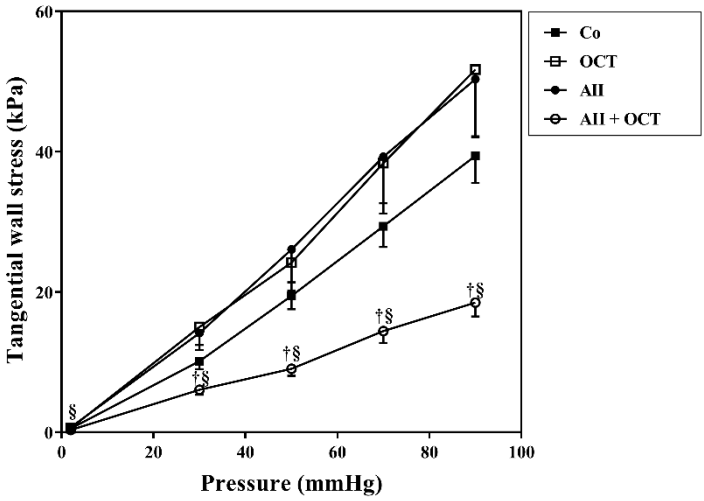

Figure 5. The tangential wall stress in the passive condition

KRUSKAL-WALLIS test at the same pressure with post hoc Dunn's test. All values are expressed in mean ± SEM. †P < 0.05 OCT vs. AII + OCT; §P < 0.05 AII vs. AII + OCT.

4.4. Vascular reactivity

The spontaneous tone of the coronary arteries harvested from Ang II acetate treated groups (AII and AII + OCT) was significantly higher than of the vessels taken from Co and OCT groups (Fig. 6). The U46619-induced constriction were significnatly smaller in the coronary arteries of the orchidectomized animals (OCT and AII + OCT groups) compared to that of the Co and AII rats (Fig. 7). The bradykinin induced relaxation was significantly smaller in orchidectomized rats (OCT and AII + OCT groups) than in Co and AII animals (Fig. 8).

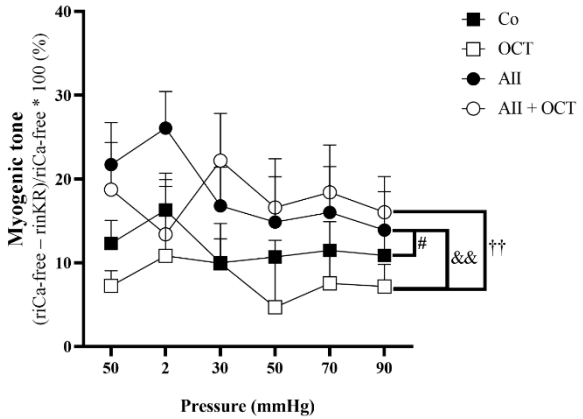

Figure 6. Spontaneous tone in nKR solution at 50 mmHg

The MIXED-EFFECTS analysis was applied to the repeated measures data (pressure curves) with post hoc Tukey's test. All values are expressed in mean ± SEM. #P <0.05 Co. vs. AII; &&P < 0.01 OCT vs. AII; ††P < 0.01 OCT vs. AII + OCT.

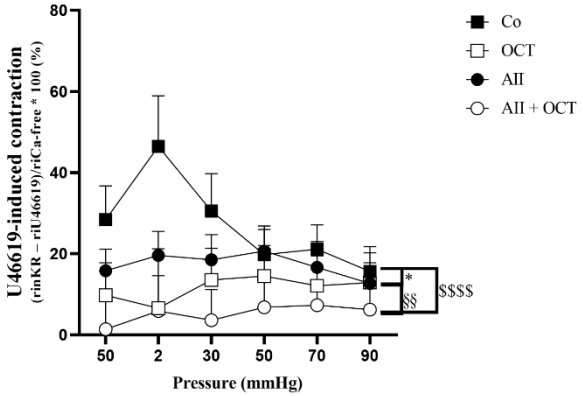

Figure 7. U46619-induced contraction at 50 mmHg

Twe-way ANOVA with post hoc Tukey's test. $P < 0.05 vs. Co; #P < 0.05 The MIXED-EFFECTS analysis was applied to the repeated measures data (pressure curves) with post hoc Tukey's test. All values are expressed in mean ± SEM. *P < 0.05 Co vs. OCT; $$$$P < 0.0001 Co vs. AII + OCT; §§P < 0.01 AII vs. AII + OCT.

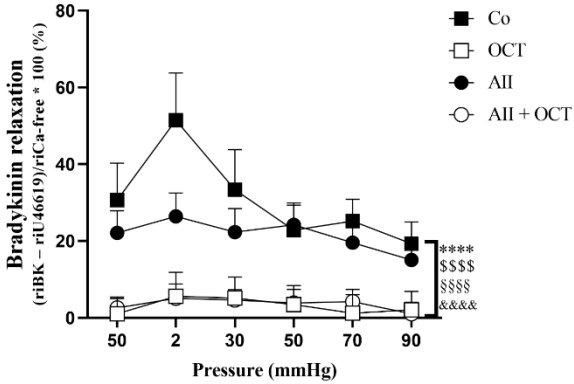

Figure 8. Bradykinin relaxation at 50 mmHg

The MIXED-EFFECTS analysis was applied to the repeated measures data (pressure curves) with post hoc Tukey's test. All values are expressed in mean ± SEM. ****P < 0.0001 Co vs. OCT; $$$$P < 0.0001 Co vs. AII + OCT; &&&&P < 0.0001 OCT vs. AII; §§§§P < 0.0001 AII vs. AII + OCT.

5. Conclusions

In our present study, we examined the effect of testosterone deficiency, and the effect of the coexistence of testosterone deficiency and hypertensive stimulus on the morphological, biomechanical, and functional adaptation of intramural coronary resistance arterioles.

In our model, we confirmed that

- as a result of hypertension, spontaneous tone increases first, even without remodeling of wall structure

Our novel results indicate, that

- testosterone deficiency decreases wall thickness
- testosterone deficiency alone results in inward hypotrophic remodeling, which persists in double noxa
- hypertensive stimulus with testosterone deficiency further reduces vessel diameter, wall thickness to lumen ratio increases and tangential wall tension decreases
- testosterone deficiency also reduces endothelial vasodilation and thromboxane agonist constriction
- in case of the coexistence of the two noxa, the spontaneous tone increases, the degree of contraction and the degree of endothelial dilation decreases, so the harmful effects caused by the two noxa add up
- thus testosterone deficiency further enhances vascular damage caused by hypertension
- however, the association of two harmful noxa does not alter what was altered by the single factor

6. Bibliography of the candidate's publications

Publications related to the thesis:

1. Jósvai, A., et al., Additive damage in the thromboxane related vasoconstriction and bradykinin relaxation of intramural coronary resistance arterioles in a rodent model of andropausal hypertension. Heliyon, 2022. 8(11): p. e11533.IF: 3.776
2. Jósvai, A., et al., Effects of Testosterone Deficiency and Angiotensin II-Induced Hypertension on the Biomechanics of Intramural Coronary Arteries. J Sex Med, 2020. 17(12): p. 2322-2330. IF: 3,802

Publications not related to the thesis:

1. Csókay, A., et al., Neurosurgical Steps and Lessons Learned in Final Separation of 3-Year-Old Craniopagus Twins. World Neurosurg, 2022. 164: p. 290. IF: 2.210
2. Vezér, M., et al., Sex differences in rat renal arterial responses following exercise training. Am J Physiol Heart Circ Physiol, 2022. 322(2): p. H310-h318. IF: 5.125
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∑IF: 32.733