Penile Histomorphometrical Evaluation in Hypertensive Rats Treated with Sildenafil or Enalapril Alone or in Combination: A Comparison with Normotensive and Untreated Hypertensive Rats

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DOI: 10.1111/jsm.12750

ABSTRACT

Introduction. Erectile dysfunction (ED) is frequently associated to hypertension and antihypertensive drugs; however, the penile morphological aspects on these situations are poorly known.

Aim. Evaluate the penile morphology of untreated hypertensive rats and rats treated with enalapril or sildenafil alone or in combination to verify the hypothesis that morphological alterations promoted by hypertension on corpus cavernosum could be ameliorated by the use of angiotensin-converting enzyme inhibitors and/or phosphodiesterase type 5 inhibitors.

Methods. Fifty male rats were assigned into five groups: normotensive rats, untreated spontaneously hypertensive rats (SHRs), and SHR treated with enalapril or sildenafil alone or in combination. Blood pressure was measured weekly. At the conclusion of the study, the rats were euthanized, and their penises were collected for histomorphometrical analysis.

Main Outcome Measures. The cross-sectional areas of the penis, tunica albuginea, and corpus cavernosum were measured. The density of the corpus cavernosum structures was quantified.

Results. Both groups of SHR rats treated with enalapril became normotensive. Untreated SHR showed no difference in penile and cavernosal cross-sectional area compared with normotensive rats; however, those rats treated with enalapril or sildenafil alone demonstrated an increase in these parameters. Rats receiving combination therapy showed no cross-sectional area differences compared with normotensive rats. Cavernosal connective tissue density was increased, while the sinusoidal spaces were diminished in untreated SHR. All treatments were effective in maintaining connective tissue density in comparison with normotensive animals. Cavernosal smooth muscle density was similar in all groups, with the exception of the combination therapy group, which demonstrated a reduction in smooth muscle.

Conclusions. Hypertension promoted structural alterations in the corpus cavernosum that may be related to ED. Enalapril- and sildenafil-treated animals had preservation of normal corpus cavernosum structure and an increase in penile and cavernosal cross-sectional area. The combination of these drugs showed less benefit than individual use.

Felix-Patrício B, Medeiros JL, Jr, De Souza DB, Costa WS, and Sampaio FJB. Penile histomorphometrical evaluation in hypertensive rats treated with sildenafil or enalapril alone or in combination: A comparison with normotensive and untreated hypertensive rats. J Sex Med 2015;12:39–47.

Key Words. Erectile Dysfunction; Hypertension; Morphology; Penis

This work was conducted at the Urogenital Research Unit, State University of Rio de Janeiro.
All authors read and approved the final manuscript.

Introduction

The persistent inability to reach or maintain penile rigidity enough for sexual intercourse, known as erectile dysfunction (ED), is the most common sexual complaint of men presenting to their physicians, with a worldwide prevalence of 10–20% [1,2]. Systemic hypertension and ED are closely intertwined diseases. Hypertension is the most commonly reported comorbidity in patients with ED. Also, ED affects as many as 68% of hypertensive men [3]. In both diseases, endothelial factors are involved, resulting in an increased smooth muscle contraction, which leads to increased vascular pressure, poor cavernosal perfusion, and inadequate intumescence [4].

In addition to this similar pathophysiology, the use of antihypertensive agents has been thought to impair normal penile erection [5]. However, evidence suggests that only certain diuretics and beta-blockers may adversely influence erectile function. Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and calcium channel blockers have been reported to have no negative effects and, on the contrary, may possibly have positive effects on erectile function [6]. ACE inhibitors have been shown to not impair erectile function in rats [7] and may improve sexual function in humans [8].

Phosphodiesterase type 5 (PDE5) inhibitors are widely used as first-line therapy for ED in normotensive and hypertensive men. As PDE5 inhibitors act by promoting smooth muscle relaxation, it has been suggested that they could ameliorate elevated arterial blood pressure by relaxing vascular smooth muscle in a manner similar to their action on corpus cavernosum smooth muscle. Some authors have shown that daily usage of PDE5 inhibitors may be beneficial in antihypertensive therapy [9,10].

Although ED may be of multiple causes [11], the structure of the corpus cavernosum is thought to play a key role in the mechanism of erection [12]. The parenchyma of the corpus cavernosum is mainly composed by collagen fibers supporting smooth muscle cells that surround the sinusoidal spaces. These spaces are filled by blood leading to penile elongation and rigidity during erection [13]. Normal proportions of these structures are required for developing and maintaining erection, and altered proportions may be related to ED [12].

The spontaneously hypertensive rat (SHR) strain was obtained by inbreeding of Wistar Kyoto rats with elevated blood pressure [14]. In these rats, blood pressure continuously increases from the fifth to the fifteenth week of life when it stabilizes in values higher than 200 mm Hg. SHR animals presents vascular alterations and thus have been the main animal model used to study hypertension and other cardiovascular-associated diseases, including ED [15]. The penis of these animals showed structural and ultrastructural alterations, with sinusoidal collagen increase and vascular wall modifications [16].

Although the morphology of the corpus cavernosum was shown to be altered by hypertension in the SHR model, with impairment of erectile function in these animals [16–18], it is not known if these morphological alterations can be prevented or reversed with the use of enalapril (an ACE inhibitor), sildenafil (a PDE5 inhibitor), or a combination of both medications. The hypothesis of this study was that morphological alterations on corpus cavernosum, promoted by hypertension, could be ameliorated by the use of ACE inhibitors and/or PDE5 inhibitors. Thus, the objective of this study was to compare, in an animal model, the penile morphology of normotensive rats, untreated SHR, SHR treated with enalapril or sildenafil alone, and SHR treated with these two drugs in combination.

Material and Methods

Experimental Design

Fifty 120-day-old male rats (n = 40 SHR and n = 10 Wistar Kyoto) were used in this experiment. The rats were maintained in an animal facility room at a temperature of 21 ± 1°C, with a controlled 12-hour light/dark cycle (artificial light, 7:00 AM to 7:00 PM). The rats received commercial food and water ad libitum. All procedures were carried out in conformity with the conventional guidelines on animal experimentation. The experimental protocols were approved by the Institutional animal experimentation ethics committee (protocol no. CEUA/051/2012).

The rats were divided into five groups of 10 animals each: (i) WKY group, composed of normotensive Wistar Kyoto rats; (ii) H group, composed of SHR; (iii) HE group, composed of SHR treated with enalapril; (iv) HS group, composed of SHR animals treated with sildenafil; and (v) HES group, composed of SHR treated with enalapril and sildenafil.

All drugs were administered orally for 40 days. Groups HE and HES received 30 mg/kg/day of enalapril (Primordium, Rio de Janeiro, Brazil)

[7,19], and groups HS and HES received 1 mg/kg/day of sildenafil (Primordium) [20]. The drugs were diluted so that each animal received 2 mL/day by gavage. The animals of groups WKY and H received 2 mL/day of saline by gavage during the treatment period.

Blood Pressure Measurement

The blood pressure measurement was used to confirm the hypertensive model and to measure the effect of the drugs on this parameter. The systolic arterial pressure was measured by tail-cuff plethysmography (Insight, São Paulo, Brazil) weekly, beginning at 90 days of age, so that by the beginning of the experiment, at age 120 days, the animals were adapted to the procedure. The mean of three consecutive measurements was used for calculation of each animal's blood pressure.

Euthanasia and Histological Procedures

At age 160 days, the rats were euthanized with an anesthetic overdose, and their penises were dissected and fixed in 4% buffered formalin. Considering a distal bone present in the rat penis, the midshaft of each organ was used for the morphological analyses. This tissue was processed for paraffin embedding and prepared in 5-μm thick sections, which were stained with picrosirius red or Masson's trichrome.

Immunolabeling was also performed with the primary antibody antiproliferating cell nuclear antigen (PCNA, product code 13–3900, Invitrogen, Camarillo, CA, USA) diluted to 1:100.

Morphological analyses were carried out with photomicrographs, captured with a digital camera (DP70, Olympus, Tokyo, Japan) coupled to a microscope (BX51, Olympus).

Morphometric Analyses

The penis of each animal was examined visually through ×20 images of cross sections stained with picrosirius red. In these bright field captured images, the areas of the penis, corpus cavernosum including its tunica albuginea, and the corpus cavernosum without the tunica albuginea were measured in square millimeters using the Image J software (version 1.45s, National Institutes of Health, Bethesda, MD, USA) with the "Free Hand" tool after calibration with a micrometric ruler. The area of the tunica albuginea was estimated by the difference in the areas of the corpus cavernosum with and without the tunica albuginea.

The surface densities (Sv) of the connective tissue, sinusoidal space, and smooth muscle of the corpus cavernosum were measured by examining cross sections stained with Masson's trichrome. For each animal, 25 photomicrographs of the corpus cavernosum were obtained under ×200 magnification. The density of each of these structures was expressed as a percentage, obtained by the point-counting method [21]. Briefly, a 100-point grid was superimposed over the images using the "grid" tool of Image J software, and each structure touched by one point was counted as connective tissue, sinusoidal space, smooth muscle, or other structure. The "cell counter" tool of Image J software was used for counting separately each structure.

The cell proliferation rate of each corpus cavernosum was quantified by the number of cells in proliferation per square decimeters. For this, slides that had been immunolabeled with the anti-PCNA antibody were used. The images were captured at ×600 magnification, and the number of immunolabeled nuclei was counted with the aid of the "cell counter" tool of the Image J software. The area of the analyzed field was measured with the "measure" tool, after calibration of the software with a micrometric ruler photomicrography.

Statistical Analyses

For each parameter, the means of H, HE, HS, and HES were compared with WKY, and the means of HE, HS, and HES were compared to H. The results were first analyzed by the Kolmogorov–Smirnov normality test. Parametric data were then compared by one-way anova with Bonferroni's post-test, while nonparametric data were compared by the Kruskal–Wallis test and Dunn's post-test. For all analysis, two-tailed tests were used. All analyses were performed with the GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, USA). Mean differences were considered significant when $P < 0.05$. All results are presented as mean ± standard deviation.

Results

Blood Pressure

At the initiation of the experiment, all animals of the SHR strain (H, HE, HS, and HES groups) had blood pressures >204 mm Hg, which was statistically higher than in the Wistar Kyoto animals (155.7 ± 20.0; $P < 0.0001$), thereby confirming the validity of the experimental model.

Table 1 Blood pressure measurements at 120 days (initiation of treatment) and 160 days of age (conclusion of treatment)

	WKY	H	HE	HS	HES	*P* value
Initial blood pressure (mm Hg)	155.7 ± 20.0	239.2 ± 33.1*	237.7 ± 12.5*	254.1 ± 37.0*	234.4 ± 12.0*	<0.0001
Final blood pressure (mm Hg)	153.4 ± 24.4	205.1 ± 24.8*	153.9 ± 21.0†	212.1 ± 6.5*	156.2 ± 11.5†	<0.0001

Data are presented as mean ± standard deviation. For each parameter, *represents statistical difference from WKY; †represents statistical difference from H. Data from normotensive rats (WKY), untreated hypertensive rats (H), and hypertensive rats receiving enalapril (HE), sildenafil (HS), or a combination of enalapril and sildenafil (HES)

At the conclusion of the study, the rats that received enalapril (HE and HES groups) had blood pressures similar to that of the WKY group. The animals of the H and HS groups remained hypertensive. The blood pressure data are reported in Table 1.

Penile Cross-Sectional Area

There was no difference in the penis cross-sectional diameter in the hypertensive (H) animals in comparison with the normotensive animals (WKY). However, the animals treated with enalapril (HE) and with sildenafil (HS) had increases in the cross-sectional area of 9% and 17%, respectively, while rats receiving the combination of these drugs (HES) demonstrated no change in penile cross-sectional area.

Corpus Cavernosum Cross-Sectional Area With and Without the Tunica Albuginea

The cross-sectional area of the corpus cavernosum with and without the tunica albuginea was similar in the WKY and H groups. The area of the corpus cavernosum without the tunica albuginea was 13% larger in the rats that received enalapril (HE group) when compared with the WKY group. The corpus cavernosum with and without the tunica albuginea in the sildenafil-treated group (HS group) increased by 12% and 10%, respectively, in comparison with the WKY group. The combination therapy group (HES group) showed no change in the cross-sectional area of the corpus cavernosum with and without the tunica albuginea in comparison with the normotensive WKY group.

Tunica Albuginea Cross-Sectional Area

The cross-sectional area of the tunica albuginea was similar in the H group and the WKY groups. The rats in the HE, HS, and HES groups did not show any significant changes in the cross-sectional area of the tunica albuginea in comparison with the WKY group. However, the HS group showed a 21% increase in the tunica albuginea cross-sectional area when compared with the H group.

Connective Tissue Density of the Corpus Cavernosum

When analyzing the connective tissue by point-counting planimetry, we observed a 13% increase in connective tissue density in the H group in comparison with WKY group. The HE, HS, and HES groups showed connective tissue density values similar to that of the WKY group (Figure 1).

Sinusoidal Space Density of the Corpus Cavernosum

The analysis of the density of sinusoidal space showed a 33% reduction in group H in comparison with the WKY group, while the density of the treated groups (HE, HS and HES) was equal to that of the WKY group (Figure 1).

Smooth Muscle Density of the Corpus Cavernosum

The density of smooth muscle in the corpus cavernosum was 23% less in the HES group in comparison with the WKY group. Groups H, HE, and HS showed densities similar to the WKY group.

Cell Proliferation

PCNA immunohistochemical staining showed a 50% greater positive cells in the H group, while values in the HE, HS, and HES groups were similar in comparison with the WKY group. All morphological results are presented in Table 2.

Discussion

Arterial hypertension is a worldwide public health condition that affects more than one-quarter of the adult population [22]. Several diseases have been associated with hypertension, including ED. In both of these diseases, endothelial dysfunction plays an important role, impairing adequate smooth muscle relaxation for the control of arterial blood pressure or for the promotion of cavernous intumescence [4].

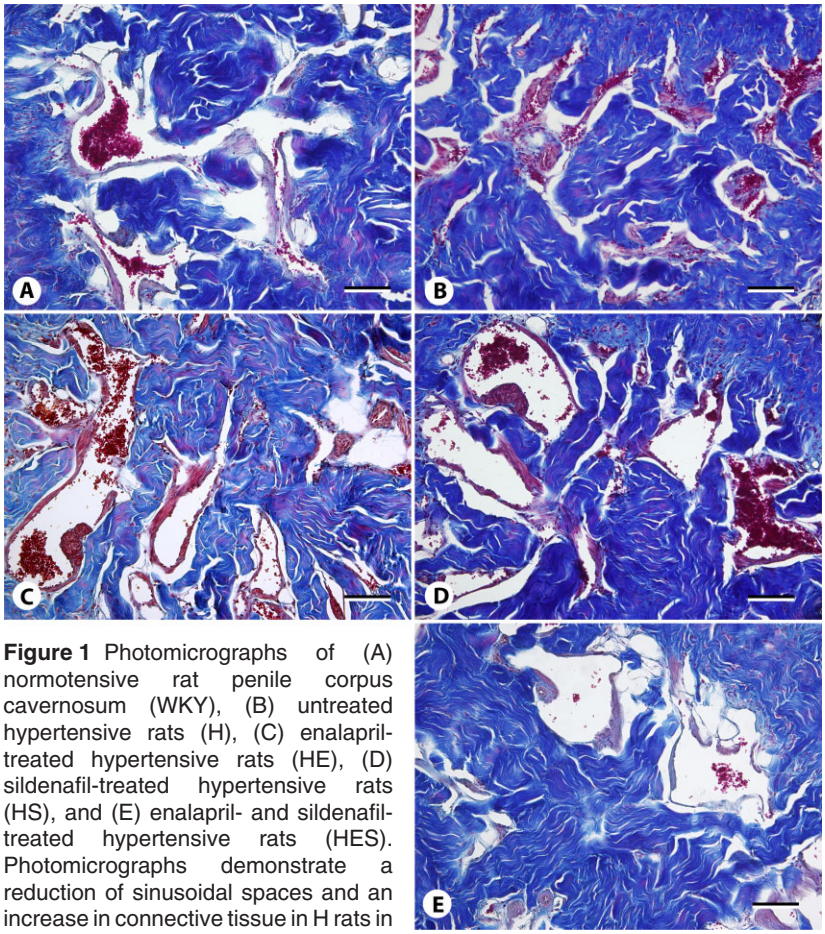

Figure 1 Photomicrographs of (A) normotensive rat penile corpus cavernosum (WKY), (B) untreated hypertensive rats (H), (C) enalapril-treated hypertensive rats (HE), (D) sildenafil-treated hypertensive rats (HS), and (E) enalapril- and sildenafil-treated hypertensive rats (HES). Photomicrographs demonstrate a reduction of sinusoidal spaces and an increase in connective tissue in H rats in comparison with other groups (WKY, HE, HS, and HES). Masson's trichrome, X200. Scale bars represent 100 μm.

SHRs are an animal model commonly used for studies of hypertension, including studies focusing on ED [23–25]. In the SHR model, it has been shown that hypertension induces morphological alterations in the corpus cavernosum with increases in both collagen and smooth muscle [18,26]. Although, an increase in smooth muscle was not observed in our study, trabecular connective tissue, composed primarily of collagen, was increased in the hypertensive rats.

An increased amount of cavernosal connective tissue is highly associated with penile fibrosis and is commonly observed in patients with ED [27]. Penile fibrosis has also been related to aging,

Table 2 Penile morphometrical data from WKY, H, HE, HS, or HES

	WKY	H	HE	HS	HES	*P* value
Penile cross-sectional area (mm^2)	7.71 ± 0.8	7.63 ± 0.6	8.42 ± 0.6*†	9.08 ± 0.5*†	7.92 ± 0.8	<0.0001
Corpora cavernosa area with tunica (mm^2)	5.19 ± 0.3	5.23 ± 0.5	5.54 ± 0.5	5.83 ± 0.4*†	5.21 ± 0.6	0.0012
Corpora cavernosa area without tunica (mm^2)	2.71 ± 0.3	2.80 ± 0.2	3.08 ± 0.2*†	3.00 ± 0.2*	2.70 ± 0.3	<0.0001
Area of the tunica albuginea (mm^2)	2.57 ± 0.1	2.40 ± 0.2	2.38 ± 0.2	2.9 ± 0.3†	2.478 ± 0.3	0.0124
Sv of the sinusoidal space (%)	25.86 ± 2.0	17.15 ± 3.3*	22.36 ± 3.7†	22.34 ± 4.5†	25.79 ± 4.5†	<0.0001
Sv of connective tissue (%)	59.40 ± 3.3	67.55 ± 2.7*	59.26 ± 6.4†	61.36 ± 7.0	62.69 ± 4.9	0.0013
Sv of smooth muscle tissue (%)	13.70 ± 2.2	14.19 ± 2.1	14.86 ± 1.7	12.87 ± 1.2	10.52 ± 1.2*†	0.0002
PCNA (cells/dm^2)	18.03 ± 3.1	27.94 ± 3.2*	19.51 ± 4.5†	23.44 ± 2.3	17.63 ± 1.5†	0.0009

Data are presented as mean ± standard deviation. For each parameter, *represents statistical difference from WKY; †represents statistical difference from H
H = untreated hypertensive rats; HE = hypertensive rats receiving enalapril; HES = combination of enalapril and sildenafil; HS = sildenafil; PCNA = proliferating cell nuclear antigen; Sv = surface density; WKY = normotensive rats

diabetes mellitus, cavernous nerve damage, chronic stress, and androgen deprivation [21,27].

Previous studies reporting increased smooth muscle content analyzed the long-term effects of hypertension on SHR corpora cavernosa [18,26]. In distinction from our study, in which the animals were killed at 5 months of age, these studies evaluated the penile histology of 8-month-old rats. Thus, it may be the case that increases in smooth muscle content develop more slowly than increases in other connective tissue components.

Hypertension has been showed to produce important alteration in cavernosal vasculature. Previous studies pointed that the vascular smooth muscle of corpora cavernosa is augmented in response to hypertension [16]. Also, hypertension leads to endothelial abnormalities by shear stress which culminates in an altered production of vasoactive substances, enhanced endothelial proliferation, and intimal permeability. These alterations can be a cause of cavernosal hypoxia which has been pointed as a cause of ED.

A low cavernosal oxygen tension is known to stimulate the expression of transforming growth factor-β1 (TGF-β1) and inhibits the prostaglandin E synthesis [28]. In cell culture studies, TGF-β1 was shown to increase by two to four folds the synthesis of collagen. Also, prostaglandin E seems to be responsible to suppress the TGF-β1-induced collagen synthesis [29]. Thus, the penile fibrosis seen on hypertensive rats in previous and in the present study could be a consequence of PGFβ1 increase resulting from the hypoxia.

Most importantly, these alterations was prevented or reversed by the therapies proposed. Antihypertensive therapy was shown to reverse or prevent the hypertensive vascular alterations as they maintain blood pressure in normal range [30]. Also, PDE5 inhibitors are thought to increase penile oxygen tension by acting in smooth muscle relaxation, also augmenting the penile blood flow [31]. These could be possible explanations for the good results found on treated groups of our study.

The amount of PCNA-positive cells in the corpora cavernosa of hypertensive animals was much higher than that seen in controls. Although these proliferating cells were not classified, one can suppose that fibroblasts, the most common cells in the rat corpora cavernosa, are involved in this process [32]. This would be consistent with the fibrosis observed in these animals. Most importantly, this parameter was not elevated in the treatment groups.

As reported by previous groups, cavernosal morphology and function are closely related, and any structural alteration may lead to, or be a consequence of, ED [12,13,21]. In the SHR model, hypertension has been related to impaired penile erection, as measured by intracavernosal pressure [17]. This finding confirms that an important relationship exists between penile morphology and function in the SHR model, as well as in other ED models. Therefore, cavernosal morphological studies in the SHR model are thought to be of interest for understanding erectile function in hypertension.

In the present study, the reduction in sinusoidal space density found in hypertensive animals may be a consequence of decreased intracavernosal pressure, as previously reported in SHR [17]. In this case, the sinusoids may be unable to compress the deep circumflex veins against the tunica albuginea, resulting in venous leak and low intracavernosal pressure [33].

All of the treated groups demonstrated normal sinusoidal space density, indicating that blood pressure control or improved smooth muscle relaxation, promoted by sildenafil, allowed the maintenance of adequate intracavernosal pressure. Treatment also appears to have prevented changes in trabecular connective tissues. The group that received only enalapril (HE) showed no evidence of penile fibrosis, while the groups receiving sildenafil (HS and HES) demonstrated results that can be considered as intermediate to that of the normotensive and untreated hypertensive rats as no statistical differences were found in these comparisons. With regard to smooth muscle content, the group that received both drugs (HES) had poor results in comparison with the other groups. In this case, it seems that the drugs may have had antagonistic effects. It has been previously shown that sildenafil and vardenafil prevent fibrosis and maintain normal smooth muscle content in two different rat models of ED [34,35]. Further investigations as to why sildenafil, alone or in combination with enalapril, was not as effective in SHR rats as compared with results in other models are necessary. Nevertheless, the groups that received the PDE5 inhibitor (HS and HES) demonstrated more closely normal cavernosal morphology than hypertensive animals without medication.

We observed that both penile and cavernosal cross-sectional areas were increased in both the enalapril (HE)- and sildenafil (HS)-treated groups. This is thought to be a positive effect of these drugs. Improved perfusion of the corpora

cavernosa in these groups, as demonstrated by the increased sinusoidal space density, may be the basis of this increase. Interestingly, the group that received combination therapy did not show this benefit, possibly again reflecting an antagonistic effect of these drugs. Further studies investigating the action of these drugs used in combination would be of interest. It is important to remember that sildenafil was used daily in our study. The on-demand use of sildenafil, alone or in combination with enalapril, may not have the same effects as seen in our study.

Overall, it appears that hypertension promotes morphological alterations in the corpus cavernosum as has been reported previously [16–18,26,36]. Enalapril, by reducing blood pressure, was able to prevent these alterations. Based on our findings, enalapril, or other ACE inhibitors, may be desirable options for treating hypertensive patients with some degree of ED that would not need PDE5 inhibitors. This is also in accordance to the literature that points ACE inhibitors as good options for the patients with ED in whom PDE5 inhibitor therapy is not indicated [5].

The use of PDE5 inhibitors to treat hypertensive patients is not recommended. However, the use of sildenafil in otherwise untreated hypertensive patients justifies interest in this group [37,38]. In our study, sildenafil maintained the morphology of the corpora cavernosa in SHR despite the persistence of high blood pressure.

Some limitation of the study should be pointed. As the penises of rats and humans are structurally different, the use of an animal model in this study may be considered a limitation. However, both species have the same structural components, and it appears that these components respond in a similar way when exposed to different experimental conditions [21,32]. Additionally, ED is commonly multifactorial, involving conditions besides hypertension, such as age, obesity, and hypogonadism [5,39]. In the SHR model, hypertension was a primary and isolated disease that does not correspond to the typical clinical setting.

Further investigation explaining the mechanisms by how the hypertension is linked to the penile alterations would be of interest. This could be an important step for specific pathway blockers, with potential new drugs development. As both ACE and PDE5 inhibitors showed beneficial effects, it is possible that there are different solutions for the same problem.

In conclusion, in our experimental model, hypertension promoted structural alterations in the corpora cavernosa that may be related to ED. Based on our morphological findings, enalapril would represent a desirable option for the treatment of hypertension in patients in whom erectile function is a concern. Sildenafil in continuous use maintained normal corpus cavernosum structure and prevented those changes observed in untreated hypertensive rats. The use of these drugs individually resulted in an improvement in penile morphology. However, the combination of these drugs was not as beneficial as was individual usage.

Take-Home Messages

Hypertension causes cavernosal morphologic modifications that may be one important link of this disease with ED. When treating hypertensive patients with ED, the use of ACE inhibitors would be a good choice. The over-the-count continuous use of sildenafil by untreated hypertensive individuals may be beneficial against the cavernosal modifications. Continuous use of sildenafil by enalapril-treated individuals may be as not benefic as its single usage when looking at penile morphology.

Acknowledgments

This study received grants from the National Council of Scientific and Technological Development (CNPq; www.cnpq.br), Foundation for Research Support of Rio de Janeiro (FAPERJ; www.faperj.br), and Coordination for the Improvement of Higher Education Personnel (CAPES; www.capes.gov.br), Brazil.

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Conflict of Interest: The author(s) report no conflicts of interest.

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References

1 NIH Consensus Conference. Impotence. NIH consensus development panel on impotence. JAMA 1993;270:83–90.
2 Albersen M, Mwamukonda KB, Shindel AW, Lue TF. Evaluation and treatment of erectile dysfunction. Med Clin North Am 2011;95:201–12.
3 Burchardt M, Burchardt T, Baer L, Kiss AJ, Pawar RV, Shabsigh A, de la Taille A, Hayek OR, Shabsigh R. Hypertension is associated with severe erectile dysfunction. J Urol 2000;164:1188–91.
4 Nunes KP, Labazi H, Webb RC. New insights into hypertension-associated erectile dysfunction. Curr Opin Nephrol Hypertens 2012;21:163–70.
5 Javaroni V, Neves MF. Erectile dysfunction and hypertension: Impact on cardiovascular risk and treatment. Int J Hypertens 2012;2012:627278.
6 Baumhäkel M, Schlimmer N, Kratz M, Hackett G, Hacket G, Jackson G, Böhm M. Cardiovascular risk, drugs and erectile function—a systematic analysis. Int J Clin Pract 2011;65:289–98.
7 Hale TM, Okabe H, Bushfield TL, Heaton JP, Adams MA. Recovery of erectile function after brief aggressive antihypertensive therapy. J Urol 2002;168:348–54.
8 Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen UU, Ückert S, Wespes E, Andersson KE, Lue TF, Stief CG. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med 2010;7:445–75.
9 Zusman RM, Morales A, Glasser DB, Osterloh IH. Overall cardiovascular profile of sildenafil citrate. Am J Cardiol 1999;83:35C–44C.
10 Chrysant SG, Chrysant GS. The pleiotropic effects of phosphodiesterase 5 inhibitors on function and safety in patients with cardiovascular disease and hypertension. J Clin Hypertens 2012;14:644–9.
11 Lue TF. Erectile dysfunction. N Engl J Med 2000;342:1802–13.
12 Costa WS, Carrerete FB, Horta WG, Sampaio FJ. Comparative analysis of the penis corpora cavernosa in controls and patients with erectile dysfunction. BJU Int 2006;97:567–9.
13 Goldstein AM, Meehan JP, Zakhary R, Buckley PA, Rogers FA. New observations on microarchitecture of corpora cavernosa in man and possible relationship to mechanism of erection. Urology 1982;20:259–66.
14 Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J 1963;27:282–93.
15 Pinto YM, Paul M, Ganten D. Lessons from rat models of hypertension: From Goldblatt to genetic engineering. Cardiovasc Res 1998;39:77–88.
16 Jiang R, Chen JH, Jin J, Shen W, Li QM. Ultrastructural comparison of penile cavernous tissue between hypertensive and normotensive rats. Int J Impot Res 2005;17:417–23.
17 Behr-Roussel D, Chamiot-Clerc P, Bernabe J, Mevel K, Alexandre L, Safar ME, Giuliano F. Erectile dysfunction in spontaneously hypertensive rats: Pathophysiological mechanisms. Am J Physiol Regul Integr Comp Physiol 2003;284:R682–8.
18 Toblli JE, Stella I, Inserra F, Ferder L, Zeller F, Mazza ON. Morphological changes in cavernous tissue in spontaneously hypertensive rats. Am J Hypertens 2000;13:686–92.
19 Hale TM, Okabe H, Heaton JP, Adams MA. Antihypertensive drugs induce structural remodeling of the penile vasculature. J Urol 2001;166:739–45.
20 Ozden E, Ozturk B, Kosan M, Tezel GG, Aki FT, Gur S, Ergen A, Ozen H. Effect of sildenafil citrate on penile weight and physiology of cavernous smooth muscle in a post-radical prostatectomy model of erectile dysfunction in rats. Urology 2011;77:e1–7.
21 De Souza DB, Silva D, Cortez CM, Costa WS, Sampaio FJ. Effects of chronic stress on penile corpus cavernosum of rats. J Androl 2012;33:735–9.
22 Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: Analysis of worldwide data. Lancet 2005;365:217–23.
23 Heijnen BF, Van Essen H, Schalkwijk CG, Janssen BJ, Struijker-Boudier HA. Renal inflammatory markers during the onset of hypertension in spontaneously hypertensive rats. Hypertens Res 2014;37:100–9.
24 Toblli JE, Cao G, Lombrana A, Rivero M. Functional and morphological improvement in erectile tissue of hypertensive rats by long-term combined therapy with phosphodiesterase type 5 inhibitor and losartan. J Sex Med 2007;4:1291–303.
25 Saito M, Ohmasa F, Dimitriadis F, Tsounapi P, Sejima T, Shimizu S, Kinoshita Y, Satoh K. Hydroxyfasudil ameliorates penile dysfunction in the male spontaneously hypertensive rat. Pharmacol Res 2012;66:325–31.
26 Toblli JE, Cao G, Casas G, Mazza ON. In vivo and in vitro effects of nebivolol on penile structures in hypertensive rats. Am J Hypertens 2006;19:1226–32.
27 El-Sakka AI, Yassin AA. Amelioration of penile fibrosis: Myth or reality. J Androl 2012;31:324–35.
28 Moreland RB. Is there a role of hypoxemia in penile fibrosis: A viewpoint presented to the Society for the Study of Impotence. Int J Impot Res 1998;10:113–20.
29 Moreland RB, Traish A, McMillin MA, Smith B, Goldstein I, Saenz de Tejada I. PGE1 suppresses the induction of collagen synthesis by transforming growth factor-beta 1 in human corpus cavernosum smooth muscle. J Urol 1995;153:826–34.
30 Chobanian AV. 1989 Corcoran lecture: Adaptive and maladaptive responses of the arterial wall to hypertension. Hypertension 1990;15:666–74.
31 Vignozzi L, Filippi S, Morelli A, Ambrosini S, Luconi M, Vannelli GB, Donati S, Crescioli C, Zhang XH, Mirone V, Forti G, Maggi M. Effect of chronic tadalafil administration on penile hypoxia induced by cavernous neurotomy in the rat. J Sex Med 2006;3:419–31.
32 Pinheiro AC, Costa WS, Cardoso LE, Sampaio FJ. Organization and relative content of smooth muscle cells, collagen and elastic fibers in the corpus cavernosum of rat penis. J Urol 2000;164:1802–6.
33 Miranda AF, Gallo CB, De Souza DB, Costa WS, Sampaio FJ. Effects of castration and late hormonal replacement in the structure of rat corpora cavernosa. J Androl 2012;33:1224–32.
34 Ferrini MG, Davila HH, Kovanecz I, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. Urology 2006;68:429–35.

35 Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. Biol Reprod 2007;76:915–23.
36 Hannan JL, Blaser MC, Pang JJ, Adams SM, Pang SC, Adams MA. Impact of hypertension, aging, and antihypertensive treatment on the morphology of the pudendal artery. J Sex Med 2011;8:1027–38.
37 Zhang K, Yu W, He ZJ, Jin J. Help-seeking behavior for erectile dysfunction: A clinic-based survey in China. Asian J Androl 2014;16:131–5.
38 Martin Morales A, Hatzichristou D, Ramon Llados J, Pascual Renedo V, Pimenidou A. Community pharmacy detection of erectile dysfunction in men with risk factors or who seek treatment or advice but lack a valid prescription. J Sex Med 2013;10:2303–11.
39 Gunduz MI, Gumus BH, Sekuri C. Relationship between metabolic syndrome and erectile dysfunction. Asian J Androl 2004;6:355–8.