Protection of Cavernous Tissue in Male Spontaneously Hypertensive Rats

Beyond Blood Pressure Control

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Male erectile dysfunction is increased in prevalence in patients with hypertension. Previous experiments from our group demonstrated morphologic changes in erectile tissue from male spontaneously hypertensive rats (SHR). The aim of the present study was to determine whether blood pressure (BP) control is enough to preserve cavernous tissue from the deleterious effect of arterial hypertension. Eight-week-old male SHR and normotensive Wistar-Kyoto rats (WKY) were studied during 6 months: Group 1 (n = 10) SHR; group 2 (n = 10) SHR with 7.5 mg/kg/d candesartan (C); group 3 (n = 10) SHR with 100 mg/kg/d atenolol (AT); and group 4 (n = 10) WKY. At the end of the experiment all the animals were killed for microscopic studies. Cavernous tissue was processed by hematoxylin-eosin, Masson’s trichrome, monoclonal anti-smooth muscle actin, and anticollagen type III. Cavernous smooth muscle (CSM) and vascular smooth muscle (VSM) from cavernous arteries and the amounts of collagen type III were evaluated. At the end of the experiment, SHR with C and AT showed similar control in BP (group 2: 131.3 ± 5.5 mm Hg; group 3: 136.5 ± 2.9 mm Hg) compared with untreated SHR (group 1: 199.6 ± 5.1 mm Hg). However, animals with C presented significantly lower values (P < .01) of CSM layer in cavernous space and VSM in cavernous arteries (P < .01), and lower amounts of collagen type III (P < .01) compared to SHR with AT and untreated SHR. We conclude that C provides a significant protective role against structural changes in vessels as well as in cavernous spaces of the erectile tissue, caused by arterial hypertension in SHR, beyond BP control. Am J Hypertens 2004;17:516–522 © 2004 American Journal of Hypertension, Ltd.

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Data from the Massachusetts Male Aging Study (MMAS) have shown a markedly high annual incidence of male erectile dysfunction (MED) in subjects between 40 and 70 years old.1 Furthermore, MED is increased in prevalence in patients with arterial hypertension and is greatly associated with cardiovascular disease.2–4

Arterial hypertension seems to produce considerable anatomic changes through the vascular tree, including cavernous vessels. In previous studies, our group reported morphologic changes in erectile tissue from male spontaneously hypertensive rats (SHR).5 These alterations were characterized by both cavernous smooth muscle (CSM) and vascular smooth muscle (VSM) hypertrophy along with cavernous tissue fibrosis. In addition, this disarrangement was highly correlated with the blood pressure (BP) level. Experiments with DOCA-salt and stroke prone spontaneously hypertensive rats have demonstrated a decreased erectile response associated with hypertension.6

Whether in hypertensive patients, it is the high BP itself or the antihypertensive therapy that constitutes a cause of MED has always been a controversial point. It is recognized that the antihypertensive group of drugs is the most commonly associated with MED.7 Moreover, among the cardiovascular drugs, both β-blockers and diuretics are recognized as a group of agents closely related to certain degree of sexual dysfunction.8 However, recently studies have reported that β-blocker therapy does not affect sexual life.9,10 Through the years, unquestionably, β-blockers have proved to be useful in reducing high BP in a large proportion of hypertensive patients, and today they are still a common therapy to initiate an antihypertensive treatment.
either as monotherapy or in combination with other antihypertensive agents. On the other hand, in the past few years, angiotensin II (Ang II) type 1 receptor blockers (ARBs) have been increasing in acceptance as a useful and effective therapy for controlling arterial hypertension in a wide range of patients. In addition, clinical studies have proven that this class of drugs is not involved in MED in patients with high BP, but it is more likely associated with improvement in sexual distress.11

Because arterial hypertension produces considerable damage in cavernous tissue, to assess whether BP control is enough to protect male genital structures, we performed the present study evaluating morphologic changes in cavernous tissue in SHR treated with a β-blocker or an ARB.

Methods
All the experiments were approved by the Hospital Aleman Ethic Committee and the Teaching and Research Committee, and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Eight-week-old inbred male spontaneous hypertensive rats (SHR) (n = 30) and normotensive Wistar-Kyoto (WKY) rats (n = 10) (Laboratory of Experimental Medicine, Hospital Aleman-Charles River Laboratories, Wilmington, MA), were housed in individual cages at a room temperature 21 ± 2°C and a 12-h light/darkness cycle (7 AM to 7 PM). Group 1 SHR (n = 10) received no treatment; group 2 SHR received candesartan (n = 10); group 3 SHR received atenolol (n = 10); and group 4 WKY rats (n = 10) were the control group.

Because candesartan cilexetil is practically insoluble in water, a suspending preparation in a 5% gum arabic solution with NaOH to increase the stability was prepared and administered to the animals in drinking water. An identical procedure was followed for atenolol. The same vehicle, containing gum arabic and NaOH, which was used for the candesartan and atenolol groups, was administered to both nontreated SHRs and control WKY rats. During 6 months, 7.5 mg/kg/d candesartan (C) and 100 mg/kg/d atenolol (AT) were administered continuously in drinking water to groups 2 and 3, respectively, whereas groups 1 and 4 received regular water with vehicle throughout the experiment. To control BP, doses of C and AT were chosen according to previous studies with hypertensive rats. All the animals were allowed to drink regular tap water and fed ad libitum standard rat chow with normal salt content throughout the experiment. To achieve adequate doses of C and AT ingested by each animal, every day the rats were weighed, the drinking consumption was recorded, and then doses were adjusted to the right proportion. At baseline and at the end of the study, blood samples were obtained for biochemical determinations. At the end of the experiment, all the rats were killed (pentobarbital 40 mg/kg body weight, injected intraperitoneally). The penises were rapidly excised, and harvested for light microscopy and immunohistochemistry studies.

BP Measurement
At baseline and at the end of the experiment, systolic BP was measured by tail-cuff plethysmography. Measurements were obtained with the rats restrained in a plastic chamber without anesthesia. A pneumatic pulse transducer positioned on the ventral surface of the tail distal to the occlusion cuff detected the return of the pulse after a slow deflation of the cuff. Cuff pressure was determined by a Pneumatic Pulse Transducer, using a programmed electro-sphygmanonometer PE-300 (Narco Bio-Systems, Austin, TX), and pulses were recorded on a Physiograph MK-I11 (Narco Bio-Systems). A minimum of three determinations were taken at each session, and the systolic BP registered was the average of the three readings.

Biochemical Procedures
After 14-h of fasting, rat blood samples were collected from the tail vein in capillary tubes at baseline, and at the end of the experiment, from the inferior cava vein before the rats were killed. Plasma glucose levels were measured by the glucose oxidase method with an automatic analyzer. Aliquots of sera were assayed for creatinine using the enzymatic UV method (Randox Laboratories Ltd., Crumlin, Northern Ireland). Serum cholesterol, triglycerides, and electrolytes were assessed according to standard methods.

Tissue Processing and Examination
Penises were removed, cut longitudinally and fixed in phosphate-buffered 10% formaldehyde (pH 7.2). The tissue samples were embedded in paraffin. Three-micron sections were cut and stained with hematoxylin-eosin, periodic acid-Schiff reagent, and Masson’s trichrome.

Immunolabeling and Light Microscopy
Immunolabeling of specimens was carried out by a modified avidin-biotin-peroxidase complex technique Vectastain ABC kit (Universal Elite, Vector Laboratories, Burlingame, CA). After deparaffinization and rehydration, the sections were washed in phosphate buffer saline (PBS), for 5 min. Quenching of endogenous peroxidase activity was achieved by incubating the sections for 30 min in 1% hydrogen peroxide in methanol. After washing them in PBS at pH 7.2 for 20 min, they were incubated with blocking serum for 20 min. Thereafter, the sections were incubated with the primary antibody, and were rinsed in PBS and incubated with Biotynilated Universal Antibody for 30 min. After washing in PBS, they were incubated for 40 min with Vectastain Elite ABC reagent, and exposed for 5 min to 0.1% diaminobenzidine (Polyscience, Warrington, PA) and 0.2% hydrogen peroxide in 50 mmol/L Tris buffer at pH 8. The α-smooth muscle actin (α-SMA) and collagen type III were quantified using anti-mouse α-SMA (Sigma Chemical Co., St. Louis, MO) monoclonal
antibodies and anticollagen type III (Biogen, San Ramon, CA) monoclonal antibodies.

Morphometric Analysis

Histologic sections were studied in each animal with an image analyzer Image-Pro Plus version 4 for Windows (Media Cybernetics, LP, Silver Spring, MD). Morphologic analyses were performed at a magnification of ×100 with the observer blind to the animal group, and the data were averaged. We evaluated the amount of: 1) cavernous smooth muscle (CSM) layer in the cavernous space (CS), expressed by the percentage of positive α-SMA immunostaining; 2) vascular smooth muscle (VSM), expressed by positive α-SMA immunostaining in vascular wall from penis arteries; and 3) collagen type III in cavernous tissue expressed as a percentage.

Statistical Analysis

Values were expressed as mean ± SD. All statistical analyses were done using absolute values and processed through GraphPad Prism, version 2.0 (GraphPad Software, Inc., San Diego, CA).

For parameters with Gaussian distribution, comparisons among groups were carried out using ANOVA; for those parameters such as histologic data with non-Gaussian distribution comparisons were performed by Kruskal-Wallis test (nonparametric ANOVA) and Dunn’s multiple comparison test. A value of $P < .05$ was considered significant.

Results

At baseline, there were no significant differences between SHR and WKY rats regarding metabolic parameters. As expected, at the end of the experiment, untreated SHR (group 1) showed a marked elevation in systolic BP relative to WKY rats (group 4). SHR given C or AT presented BP records close to the control WKY rats, as illustrated in Fig. 1. In addition, serum creatinine was also significantly ($P < .01$) higher in untreated SHR (64.5 ± 3.8 μmol/L) in comparison with the other groups, which presented similar values with respect to each other (SHR + C: 54.6 ± 2.3 μmol/L; SHR + AT: 55.1 ± 2.2 μmol/L; WKY: 54.5 ± 1.8 μmol/L). On the other hand, at the same time, no significant changes in serum glucose, cholesterol, and triacylglycerides were observed between the groups.

Microscopic examination of the cavernous tissue revealed that, although untreated SHR presented a significant increased amount of α-SMA in arteries and in the CSM layer in CS with respect to control WKY rats, SHR treated with C showed significantly lower values, which were also similar to the WKY rats group (Figs. 2, 3, and 4).
In contrast, SHR given AT, despite reducing both \(\alpha\)-SMA in arteries and in the CSM layer in CS, presented no statistical significant differences with respect to untreated SHR, as shown in Figs. 2, 3, and 4.

The amount of collagen type III in cavernous tissue was significantly higher in untreated SHR when compared with WKY rats. However, SHR, which received C, presented a remarkable reduction in the percentage of collagen type III, which was also lower than the one that SHR with AT showed, as illustrated in Figs. 2 and 5.

**Discussion**

In the present study, SHR showed a remarkable VSM hypertrophy pattern of the cavernous arteries as well as an increase of the smooth muscle layer in the CS, along with a higher amount of collagen type III, which were very different from those showed by normotensive control WKY rats. Both C and AT treatments achieved a satisfactory BP control in SHR at the end of the experiment. However, only those animals that had received C presented a significant protection on cavernous tissue. The beneficial effect of C was characterized by a substantial reduction in CSM layer in the CS, as well as in VSM in vessels through the erectile tissue. Moreover, extracellular matrix (ECM) expansion, expressed by the amount of collagen type III, was also reduced in the cavernous tissue in these animals.

Penile erection is a complex series of integrated control systems that involve endothelial cells, VSM cells, fibroblast, ECM, and nerves. Any shift in the balance of this control system either toward trophic responses such vascular hypertrophy, focal fibrosis, or increased production of ECM results in MED.

Reduction in the blood flow to the erectile tissue is believed to be the most frequent organic cause of MED. In addition, in experimental models of vasculogenic erectile dysfunction, the hemodynamic contribution of reduced arterial inflow and perfusion pressure to erectile dysfunction has been well established.

One of the principal mechanisms involved in MED is an increase in the tone or contractility of the CSM layer in the CS and penile arteries that impedes the modulation of penile blood flow by physiologic regulators such as nitric oxide (NO). Relaxation of the CSM causes increased compliance of the CS, leading to penile engorgement and erection.
Various studies have reported disarrangement in the CSM cells due to the aging process or corresponding to pathologic modifications as a consequence of specific risk factors related to the vascular system. In extracavernosal segments of the vascular bed, the tone and contractility of VSM and then the regulation of local blood flow mostly depend on a balance between Ang II and NO. However, the sympathetic nervous system (SNS), which is commonly activated in arterial hypertension, could be a partner in this mechanism. Recent studies have demonstrated the adrenergic stimulation capacity to directly modulate both transforming growth factor-β (TGF-β), one of the essential cytokines involved in the fibrotic process and ECM protein synthesis in VSM. Therefore, it should be congruent to think that the reduction of SNS activity by β-blockers will result in a better control in vascular remodeling, tissue fibrosis, and, as an outcome, cavernous vascular bed protection. Nevertheless, convincing information suggests that β-blockers fail to modify the deleterious process of high BP in the vasculature of hypertensive patients. In the present study, SHR treated with AT, despite showing a satisfactory control in BP, presented no significant difference in VSM or in CSM hypertrophy pattern in comparison with untreated SHR. Moreover, collagen type III accumulation in cavernous tissue was similar between these two groups. This indicates that β1-receptor blockade with AT is not enough to protect cavernous and vascular structures against high BP in cavernous tissue.

In the past years, experimental as well as clinical studies have demonstrated that corpus cavernous produces and secretes physiologically relevant amounts of Ang II and that the local renin-angiotensin system (RAS) is involved in the regulation of CSM tone through Ang II receptor subtype 1. Angiotensin II has been identified in corpus cavernosum, especially in endothelial cells and CSM, where its concentration is several times higher than plasma levels and aortic or mesenteric arteries. Local angiotensin-converting enzyme regulates CSM tone in a paracrine fashion throughout Ang II production, which in turn stimulates contraction of CSM by the Ang II type 1 receptor. Intra-cavernosal infusion of Ang II produces contraction of CSM and terminates spontaneous erection in anesthetized dogs, whereas administration of an ARB results in smooth muscle relaxation and, consequently, erection.

A number of studies has demonstrated that Ang II through the AT1 receptor induces VSM cell proliferation and hypertrophy and has an essential role in ECM expansion. On the other hand, ARBs have shown a clear
benefit by controlling VSM growth and modulating ECM protein synthesis in different tissues. Characteristics of CSM are similar to those of VSM. Therefore, potential pathophysiologic projections, particularly for the use of selective Ang II blocking agents, acquired importance. Because both CSM cell hypertrophy and hyperplasia, by producing a substantial impediment to achieve the complete compliance of the CS and in addition by increasing the surrounding connective tissue expressed by remarkable amount in collagen type III, can contribute considerably to make normal penile tumescence difficult. The control in both CSM growth and ECM modulation in cavernous tissue play a relevant role in this setting. In our study, SHR that had received C displayed a notorious and significant reduction in CSM, VSM, and collagen type III in cavernous tissue. These findings suggest that C can control the remodeling process in these vascular structures and prevents cavernous tissue fibrosis. Interaction against local RAS by ARBs may improve penile erectile dysfunction in patients with arterial hypertension, throughout several mechanisms of action: 1) by reducing VSM and CSM hypertrophy in cavernous tissue; 2) by modulating collagen accumulation in cavernous tissue; and 3) by increasing penile blood flow through local generation of NO, which produces CSM relaxation in CS.

In conclusion, despite the possible limitation in the present study because we have not included functional experiments, we believe that our results demonstrate that AT1 receptor blockade produces a substantial benefit with respect to morphologic abnormalities in cavernous tissue caused by arterial hypertension in this animal model. Taking into account, the cardinal role of local RAS in the regulation of erectile function, the data shown in our study provide some evidence suggesting a potential additional help of ARBs in the management of patients with high BP.

Because hypertensive patients with cardiovascular complications such as coronary artery diseases or heart failure are more susceptible to developing MED, and in spite of the fact that the primary goal of antihypertensive treatment is to lower BP, the right choice of adequate therapy increases in importance to preserve a better quality of life.

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