Effect of an angiotensin II receptor blocker and a calcium channel blocker on hypertension associated penile dysfunction in a rat model

Shogo Shimizu1, Panagiota Tsounapi2, Masashi Honda3, Fotios Dimitriadis4, Keisuke Taniuchi1, Takahiro Shimizu1, Keiji Inoue4, and Motoaki Saito5

1 Department of Pharmacology, Kochi Medical School, Kochi University, Nankoku, Japan; 2 Division of Urology, Tottori University School of Medicine, Yonago, Japan; 3 B’Urologic Department, Papageorgiou General Hospital, School of Medicine, Aristotle University, Thessaloniki, Greece; and 4 Department of Urology, Kochi Medical School, Kochi University, Nankoku, Japan

(Received 18 April 2014; and accepted 21 April 2014)

ABSTRACT

Possible effect of olmesartan, an angiotensin II receptor blocker (ARB), or nifedipine, an L-type calcium channel blocker, on penile dysfunction in the spontaneously hypertensive rat (SHR) was investigated in this study. Twelve-week-old male SHRs were treated with olmesartan (1 or 3 mg/kg, per orally (p.o.)) or nifedipine (30 mg/kg, p.o.) once a day for 6 weeks. Wistar rats and SHRs with vehicle treatment were used as controls. Penile cGMP and malondialdehyde concentrations, and mRNA levels of endothelial and neuronal NO synthase (eNOS and nNOS) were measured. Penile function was evaluated by organ bath studies with norepinephrine-induced contractions and acetylcholine-induced relaxations. The SHR showed significantly increased blood pressure, decreased cGMP concentrations, increased malondialdehyde concentrations, decreased eNOS and nNOS mRNA levels, norepinephrine-induced hyper-contractions, and acetylcholine-induced hypo-relaxations in the penile tissue compared to the Wistar rat. Both nifedipine and olmesartan significantly decreased blood pressure, decreased cGMP concentrations, increased malondialdehyde concentrations, decreased eNOS and nNOS mRNA levels, norepinephrine-induced hyper-contractions, and acetylcholine-induced hypo-relaxations in the penile tissue compared to the Wistar rat. Both nifedipine and olmesartan significantly decreased blood pressure, increased cGMP and normalized the hyper-contractions and hypo-relaxations observed in the SHR group. However, not nifedipine but olmesartan improved the malondialdehyde concentrations and increased mRNA levels of eNOS and nNOS in the penis. Our results indicate that the hypertension-associated penile dysfunction might be treated with ARBs such as olmesartan better than calcium channel blockers, such as nifedipine.

Address correspondence to: Motoaki Saito, MD, PhD
Department of Pharmacology, Kochi Medical School, Kochi University, Nankoku 783-8505, Japan
Tel: +81-88-880-2325, Fax: +81-88-880-2328
E-mail: saitomo@kochi-u.ac.jp

Hypertension induces vascular remodeling, pathological changes in the penis, and subsequent diminished blood supply to the penile tissue. In addition, hypertension represents one of the major risk factors for the development of vasculogenic erectile dysfunction (ED) (16, 28). ED frequently appears in hypertensive patients than in normotensive individuals (10). The extent of ED directly depends on the degree and time of hypertension (8). Clinical studies show that approximately 30% of hypertension patients have ED compared to 9.6% in the common population (12, 28). Thus, antihypertensive therapies in hypertension-related ED patients are necessary in order to reduce the blood pressure and further protect the cavernous tissue. However, some of these therapies did not always improve the symptoms of ED in hypertensive patients (22, 23).

In clinical studies, the older antihypertensive drugs, i.e., diuretics and β-adrenoreceptor blockers, may exert detrimental effect on ED. On the other hand, more recent drugs have neutral (calcium channel blockers and angiotensin-converting enzyme (ACE) inhibitors) or favorable (angiotensin II type 1 receptor (AT1R) blockers (ARBs)) effects (11, 22). These data suggest that blood pressure control may not be the only treatment strategy to improve erec-
tile function. Previous studies indicate that ARBs have an additional benefit beyond blood pressure reduction (25). The renin-angiotensin system (RAS) has an important role in the ED regarding the remodelling process (27). Angiotensin II, an active product of the RAS, induces various physiological action, vasoconstriction, and endothelial dysfunction via the increased production of reactive oxygen species (ROS) and eicosanoids (9). Angiotensin II contributes to the vascular remodeling in the penis, resulting in the hypertension-related ED. Interestingly, cavernosal plasma angiotensin II levels in ED patients are higher than those in control men (5).

The spontaneously hypertensive rat (SHR) has been widely used as the hypertension-derived ED model. The model exhibits the substantial morphological changes such as vascular remodeling on penile tissue, and diminished blood flow to the erectile tissue (6, 7, 36). Also, hypertrophy of corpus cavernous smooth muscle and vascular smooth muscle has been confirmed in the SHR (32). In previous studies, the protective effect of ARB, such as losartan and candesartan, was already investigated in the penis in the SHR (9, 25). Olmesartan medoxomil (olmesartan) is a newer ARB than losartan, and more effective to antagonize the AT1R (2). Olmesartan has a rapid and long lasting inhibition of angiotensin II-induced hypertensive response. In the present study, the effect of an ARB olmesartan was investigated in hypertension-related penile dysfunction in the SHR. Moreover, to investigate the difference between antihypertensive drugs, we compared the effect of olmesartan to that of a calcium channel blocker nifedipine in the SHR.

**MATERIALS AND METHODS**

**Animal model.** The animal protocol was approved by the Tottori University Committee for Animal Experimentation and the experiments were performed according to the Principles of Laboratory Animal Care (National Institute of Health guideline; publication no. 86-23, revised 1984). Six-week-old male Wistar rats and SHRs were purchased from SLC (Shizuoka, Japan). Upon reaching the age of 12 weeks, the rats were divided into five groups (n=8 in each group): age-matched Wistar rats treated with vehicle (0.5% methylcellulose) per orally (p.o.) (Cont); SHRs treated with vehicle, p.o. (SHR); SHRs treated with olmesartan at a daily dose of 1 or 3 mg/kg, p.o. (SHR+Olm1 or SHR+Olm3); and SHRs treated with nifedipine at a daily dose of 30 mg/kg, p.o. (SHR+Nif). After six weeks of treatment with olmesartan or nifedipine, blood pressure and heart rate were measured by the tail-cuff method (BP-98A-L; Softron, Tokyo, Japan) without anesthesia (13, 29). Subsequently, the rats were sacrificed with an overdose of sodium pentobarbital (60 mg/kg, i.p.). The isolated penile tissues were used in organ bath experiments, or stored at −80°C for measurements of tissue cGMP and malondialdehyde (MDA) concentration, and expressions of endothelial NO synthase (eNOS) and neuronal NOS (nNOS) mRNAs.

**In vitro organ bath experiments.** Functional studies were performed according to our previous reports (26, 30). In short, the tunica albuginea was removed from penile tissues and then approximately 1.5 mm × 5 mm strips of corpus cavernosum were prepared. Each strip was suspended on a wire hook in an organ bath (30 mL) containing Krebs-Henseleit solution, and bubbled with 5% CO2 and 95% O2 (37°C). One hook was suspended from a transducer (type 45196A; San-eti Instruments, Tokyo, Japan), and the lower hook was fixed to a plastic support leg to a micrometer (Mitutoyo, Tokyo, Japan). Each strip was equilibrated unstretched for 30 min. A load of 1.0 g was applied to each strip by micrometer adjustment. Thirty minutes later, the load was readjusted to this level of 1.0 g. Changes in the tone were recorded by a force transducer on a personal computer (Macintosh G3; Apple Computer, Cupertino, CA) by use of Chart v 3.6.9 software and a PowerLab/16sp data acquisition system (AD Instruments, Castle Hill, Australia). Following a 30-min period of equilibration, the strips were exposed to 100 mM KCl. In the penile strips, the contractile response to norepinephrine (1 × 10⁻⁶ to 3 × 10⁻⁸ M) was determined cumulatively. After a 30-min washout period, propranolol (1 × 10⁻⁶ M) was added to prevent the involvement of β-adrenoceptor-mediated relaxation. The endothelium-mediated relaxation was measured as the concentration-response curve to acetylcholine (1 × 10⁻⁸ to 3 × 10⁻⁷ M) in strips precontracted with the submaximal dose of norepinephrine. In each strip, the concentrations of submaximal contraction were determined and were approximately from 1 × 10⁻⁶ to 1 × 10⁻⁵ M.

**cGMP concentrations in the rat penile tissue.** Enzyme-linked immunosorbent assay was used to measure cGMP concentrations in the corpus cavernosum according to the manufacturer’s instructions (cGMP Direct Immunoassay Kit; BioVision, Mountain View, CA). The protein concentrations of the super-
natant were detected using a Protein Assay Rapid Kit Wako kit (Wako Pure Chemical, Osaka, Japan).

**Measurements of MDA levels in the penile tissue.** In order to investigate whether hypertension induces oxidative damage in the penile tissue, the concentrations of MDA, a marker of lipid peroxidation, were measured in the penile tissue using a commercially available kit (NWLSM TM Malondialdehyde Assay; Northwest Life Science Specialties, LLC, Vancouver, WA) according to manufacturer’s instruction.

**Real-time polymerase chain reaction (PCR) for quantification of eNOS and nNOS mRNAs.** eNOS and nNOS mRNAs in the penis were measured by real-time PCR method. Real-time PCR was performed according to our previous reports (26). The reverse transcriptase (RT) mixture (20 μL) containing 1 μg of total RNA was prepared and subsequently incubated at 37°C for 60 min. Fifteen micro liters of the mixture were used for real-time PCR, which was performed using a Light Cycler system with a LightCycler-FastStart Hybridization Probe kit (Roche Diagnostics, Tokyo, Japan). The primers and probe sequences specific to the gene of eNOS (GeneBank Accession: AJ011116), nNOS (GeneBank Accession: NM_052799) and β-actin (GeneBank Accession: NM_031144) were used according to our previous reports (26). The primer and probe of the β-actin were from the LightCycler-Primer/Probe Set (rat), and was used as the internal standard. A total of 5 μL of cDNA solution was used for the sample.

**Data analysis and statistical analysis.** The E_max and EC_{50} values were obtained by a Macintosh computer (G3) loaded with Chart v3.6.9 software and a PowerLab/16sp data acquisition system. The data for the contractions induced by norepinephrine were normalized by the contractions induced by the cross-sectional area and also contractile data were calculated as grams of active force per cross-sectional area in square millimeters (26, 30). The cross-sectional area was calculated using the following equation: cross-sectional area = weight/(length × 1.05), where 1.05 is the assumed density of the muscle (26, 30). The expressions of eNOS and nNOS mRNAs were quantified according to the expression of β-actin mRNAs in the experimental rat penile tissue. A statistical comparison of differences between groups was performed using analysis of variance and Fisher’s multiple comparison tests. P < 0.05 was regarded as the level of significance.

**Drugs and chemicals.** Olmesartan was kindly provided by Daiichi-Sankyo Pharmaceutical Co. Ltd (Tokyo, Japan) and nifedipine was purchased from SIGMA-ALDRICH (St Louis, MO). All other chemicals (reagent grade) were commercially available.

**RESULTS**

**General features of the animal groups**
The SHR demonstrated significantly decreased body weight, penile weight and heart rate compared to the control at the age of 18 weeks. In the same manner, the treated SHR animals with olmesartan did not alter these parameters. On the other hand, while the treated SHR with nifedipine did not alter the body weight and penile weight, they showed partially increase heart rate compared to the vehicle treated SHR. The systolic, mean and diastolic blood pressures were significantly higher in the SHR group compared to the control group. Treatment with olmesartan significantly decreased all the above parameters compared to the SHR in a dose-dependent manner. Treatment with nifedipine proved to be a more effective drug to decrease the blood pressure compared to the SHR. All general features of the animal groups are presented at Table 1.

**Functional in vitro organ bath studies**
In Table 2, the contractile responses and the relaxation responses of the rat penile tissue are presented. The E_max values for contraction induced by norepinephrine and normalized by cross-sectional area in the SHR were significantly increased compared to the ones in the control group. Treatment with olmesartan inhibited this hyper-contractility compared to the SHR in a dose-dependent manner (Table 2). Also nifedipine significantly decreased the hyper-contractions compared to the SHR. However, there were no significant differences of the EC_{50} values among any of the groups. The contractile forces induced by 100 mM KCl showed no significant differences of these values between any of the groups (data not shown). The relaxation of the norepinephrine-precontracted penile tissues obtained from all groups was produced in a dose-dependent manner. The relaxation was markedly reduced in the SHR group in comparison with the control group (Table 2). Treatment with higher dose of olmesartan and nifedipine significantly recovered the attenuated relaxation.

**cGMP concentrations in the rat penile tissue**
Penile cGMP concentrations in the rat are presented
tially increased the eNOS and nNOS mRNA levels compared to the SHR, as there was not any significant difference compared with either the control or SHR groups (Fig. 2).

**DISCUSSION**

The present study is the first report, which investigated the effect of olmesartan on the hypertension-associated ED. The present data showed that treatment with olmesartan and nifedipine significantly ameliorated the blood pressure, norepinephrine-induced hyper-contractions and acetylcholine-induced hyporelaxations of the corpus cavernosum, and the penile cGMP concentrations in the SHR. However, treatment with olmesartan but not nifedipine ameliorated the MDA concentrations and the expressions of eNOS and nNOS mRNAs in the SHR. These findings suggest that treatment with olmesartan could ameliorate hypertension-related erectile damage better than treatment with nifedipine.

It is known that there is a decrease in the erectile...
function with an impaired endothelium-dependent relaxation and an extracellular matrix remodeling in the SHR corpus cavernosum (24, 34). In the present study, we observed both norepinephrine-induced hyper-contraction and acetylcholine-induced hypo-relaxation in the corpus cavernosum in the SHR, which were improved by treatment with olmesartan and nifedipine. The functional impairment of the erectile tissue in the SHR is attributed to the increased smooth muscle contraction, and the damaged relaxation induced by NO via the increasing of oxidative stress levels (24, 34). In the penis, NO is the principal regulator of the cavernosal smooth muscle relaxation, leading to penile erection. Furthermore, eNOS and nNOS are important factors for penile erection to produce NO from L-arginine in the corpus cavernosum. The impairment of NOS and NO availability should lead to develop ED (30, 36). Mazza et al. reported that treatment with an ARB, candesartan, improved cavernous sinusoidal eNOS and acetylcholine-induced hypo-relaxation in the SHR (25).

Angiotensin II is locally produced and secreted from penile endothelial cells and smooth muscle cells (18). Angiotensin II increases NADPH oxidase-dependent ROS production, followed by the stimulating RhoA/Rho kinase-signaling pathway, and decreasing the eNOS activity via the activation of the AT1R. These responses lead to an increase in the cavernosal smooth muscle contraction and the penile erectile tissue damage, resulting in the development of hypertension-associated ED (14–16). Pre-

---

**Fig. 1** cGMP concentrations and MDA concentrations in the rat corpus cavernosum. Data are shown as mean ± SEM of six to eight separate determinations in each group. Cont: 18-week-old Wistar rats (control) treated with vehicle, p.o. once a day for 6 weeks; SHR: 18-week-old spontaneously hypertensive rats treated with vehicle p.o. once a day for 6 weeks; SHR+Olm1: 18-week-old SHRs treated with olmesartan 1 mg/kg, p.o. once a day for 6 weeks; SHR+Olm3: 18-week-old SHRs treated with olmesartan 3 mg/kg, p.o. once a day for 6 weeks; SHR+Nif: 18-week-old SHRs treated with nifedipine 30 mg/kg, p.o. once a day for 6 weeks.

* Significantly different from the Cont group (P < 0.05). # Significantly different from the SHR group (P < 0.05).

**Fig. 2** eNOS and nNOS mRNA levels in the rat corpus cavernosum. Data are shown as mean ± SEM of six separate determinations in each group. Treatment with both doses of olmesartan partially increased the mRNA levels of eNOS and nNOS mRNA in the penis compared to the SHR group, but treatment with nifedipine did not increase the eNOS and nNOS mRNA levels. There was not any significant difference between Cont and SHR+Olm1 (P = 0.797) or SHR+Olm3 (P = 0.848) groups in the nNOS mRNA levels. * Significantly different from the Cont group (P < 0.05).
viously, a study from our laboratory indicated that chronic treatment with Rho kinase inhibitor hydroxyfasudil ameliorated the hypertension-derived dysfunction of NO-induced relaxation in corpus cavernosum smooth muscle in the SHR. The possible mechanism of the hydroxyfasudil-induced effects may be the inhibition of RhoA/Rho kinase pathway, thereby activating NO-cGMP pathway (30).

Nifedipine and amlodipine, L-type calcium channel blockers, are widely used as the important drugs in the treatment of hypertension and coronary heart diseases (21). Calcium channel blockers are known to induce relaxation effect on the corpus cavernosum in vitro study (20). Toblli et al. investigated the effects of an ARB losartan and a calcium channel blocker amlodipine on the SHR penis (33). The administration of losartan and amlodipine had a similar blood pressure control, but only losartan significantly improved hypertension-induced structural changes in the vessels and cavernous spaces of the penile tissue (33). It has been also reported that treatment with another ARB irbesartan reduced the vascular and cavernosal oxidative stress, and improved the endothelial function of the corpus cavernosum in cholesterol-fed ApoE−/− mice with atherosclerosis (4).

In another experimental study, treatment with amlodipine did not change the level of oxidative stress marker (thiobarbituric acid reactive substance) in corpus cavernosum in the SHR (35). These data are in agreement with our study that treatment with ARBs reduces oxidative stress in the penile tissue. Yono et al. demonstrated that nifedipine had no significant effects on eNOS and nNOS mRNA levels in the penis in the SHR (36). In our study, treatment with nifedipine had no change in the eNOS and nNOS mRNA levels, while the treatment significantly increased the penile cGMP which is the second messenger of NO derived by NOS. The cGMP reduces intracellular calcium levels, resulting in the vascular smooth muscle relaxation. There are some evidence that nifedipine increases cGMP production in the rat ventricular papillary muscle (31) and vascular smooth muscle (19). Additionally, it has been reported that nifedipine augments cGMP production through the activation of cardiac soluble guanylyl cyclase as a negative inotropic effect but does not affect the NOS expression (31). Furthermore, it has been demonstrated that treatment with amlodipine significantly increased nitrate/nitrite and cGMP level in the plasma and corpus cavernosum in the SHR (1, 35). Taken together, nifedipine and other L-type calcium channel blockers may be able to increase cGMP production in the penis independently of NOS expression. It may be one of the reasons that nifedipine ameliorated the hyper-contractions and hyporelaxations in the corpus cavernosum in the SHR.

ARBs are commonly used as antihypertensive therapy. Much clinical evidence showed that the inhibition of RAS has beneficial effects on erectile function and sexual activity without significant negative effects (3, 10, 17). Taking into account all this information, the use of olmesartan or other ARBs as the drug of first choice in hypertensive patients with ED might be recommended. However, further basic and clinical studies with each ARB are required to understand the class and the drug effect, and effective type of ARB for hypertensive patients with ED.

In conclusion, olmesartan ameliorated hypertension-related dysfunction in the corpus cavernosum smooth muscle in the SHR through the improvement of the blood pressure and NOS activity, and on the other hand by decreasing the oxidative stress levels.

Acknowledgements

This study was supported by grants in aid from the Japan Society for the Promotion of Science (#24592431, 20591880).

REFERENCES


