Characterization of an Animal Model of Postmenopausal Hypertension in Spontaneously Hypertensive Rats

Lourdes A. Fortepiani, Huimin Zhang, Lorraine Racusen, L. Jackson Roberts II, Jane F. Reckelhoff

Abstract—Blood pressure (BP) increases in postmenopausal women. The mechanisms responsible are unknown. The present study was performed to characterize a model of postmenopausal hypertension in the rat and to determine the role that oxidative stress may play in mediating the postmenopausal hypertension. Spontaneously hypertensive rats were ovariectomized (ovx) or left intact (PMR) at 8 months and were aged to 18 months. These animals were compared with young females (YF; 4 or 8 months of age) and old males (18 months) for some measurements. Estradiol levels were decreased in PMR rats to levels not different from YF rats in proestrous or from old males. BP increased progressively with age in PMR rats but not in ovx or male rats, such that the gender difference in hypertension disappeared by 18 months. Glomerular filtration rate was lower in ovx and PMR rats than in YF rats. Renal plasma flow and renal vascular resistance were similar between YF and ovx rats, but lower and higher, respectively, in PMR rats. Serum testosterone increased by 60% in ovx rats and 400% in PMR rats compared with YF rats. Plasma renin activity also increased in PMR rats but not in ovx rats. Chronic treatment (for 8 months beginning at 8 months of age) of PMR rats with vitamins E and C, but not tempol, resulted in a significant reduction in BP and excretion of F2-isoprostanes. In contrast, tempol, but not vitamins E and C, reduced BP in old males. These data suggest that the PMR rats, but not ovx rats, may be a suitable model for the study of postmenopausal hypertension, and that oxidative stress plays a role in the increased BP. 

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Key Words: women ■ menopause ■ oxidative stress ■ hormones ■ renin-angiotensin system ■ nitric oxide

Blood pressure (BP) increases after menopause in women such that the prevalence of hypertension becomes higher in women than in men.1 The postmenopausal increase in BP does not occur as soon as the ovary stops producing estradiol, but occurs over a period of 5 to 10 years. The mechanisms responsible for the postmenopausal increase in BP are not known.

Many factors have been suggested to play a role in the increased BP in postmenopausal women (PMW). For example, activation of renin-angiotensin system has been implied by data showing that plasma renin activity (PRA) was increased in PMW compared with premenopausal women.2 An increase in angiotensin (Ang) II would not only cause vasoconstriction but also influence sodium reabsorption to increase BP. Another factor that could impact BP is NO. Oxidative stress has also been shown to be increased in PMW.3 Ang II is known to cause increases in superoxide production,4 and NO is scavenged by superoxide.5,6 This is a mechanism by which the renin-angiotensin system and NO could interact to increase BP. Furthermore, oxidants, such as peroxynitrite, which is formed from superoxide and NO, has been shown to cause increases in vasconstrictors and reductions in vasodilators.7

One problem with the study of postmenopausal hypertension has been the lack of a suitable animal model. Nonhuman primates, sheep, rabbits, mice, and rats have all been used as models of menopause.8 However, to our knowledge, there has not been a model used for the study of postmenopausal hypertension. We present here the characteristics of a rat model of postmenopausal hypertension, the aging female spontaneously hypertensive rat (SHR), which is noncycling, has low serum estradiol, and has increased BP compared with that of the premenopausal state. We also examined the role that oxidative stress may play in mediating the hypertension. Because young male SHR exhibit oxidative stress, we hypothesized that the aging female would as well, and that antioxidant treatment would reduce BP.

Methods

Rats

Female SHR, aged 4 and 8 months, were obtained from Taconic Farms (Germantown, NY). Male SHR—4, 8, and 18 months—from the same vendor were also used for some comparisons. The protocols met National Institutes of Health guidelines and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.
Characterization of Postmenopausal Hypertension

Some of the 8-month-old females (n=11) were ovariectomized (ovx) by the vendor before shipment. The 8-month-old intact and ovx females were aged to 18 months. While aging, SHR were maintained on standard rat chow (Teklad, Harlan SD) and tap water in an environment with 12-hour/12-hour light/dark cycle. At the time of study, there were 3 groups of female rats: group 1, young controls (4 months; n=6); group 2, 18-month-old ovx rats (n=10); and group 3, 18-month-old postmenopausal rats (PMRs; n=8). For these studies, the following were measured: protein excretion, serum estradiol and testosterone, mean arterial pressure (MAP) and renal function, and PRA; plasma F2-isoprostanes were measured in group 1 and group 3. For comparison of MAP, male SHR (n=6 to 10 per group) were used.

Chronic Vitamin E and C and Tempol

Female and male SHR were obtained at 8 months of age. The rats from each sex were divided into 3 groups (n=6 to 7/group): controls; rats given vitamin E (5000 ppm/kg chow) and vitamin C (100 mg/kg/d) in drinking water; and rats given tempol (1 g/L) in the drinking water. All rats were treated for 8 months. We have used vitamin E chow previously in aging male rats, and chronic treatment (9 months) resulted in reductions in oxidative stress, as measured by reductions in kidney F2-isoprostanes, thiobarbituric acid reactive substances, and induction of heme-oxygenase.1 Vitamins E and C were both used because vitamin C is capable of regenerating vitamin E radical after its interaction with reactive oxygen species.10,11 In these studies, MAP was measured in anesthetized rats. Excretion of F2-isoprostanes was also measured.

Vaginal Smearing

Daily vaginal smears were performed on females to determine the stage of their estrous cycle and were correlated with serum estradiol levels. Estradiol values were subdivided for young females according to the stage of the estrous cycle: diestrous (n=6), proestrous (n=5), or estrous (n=10). Old female and ovx SHR were also subjected to vaginal smears, beginning at 8 months of age. Cessation of cycling was defined as continuous estrus for 8 weeks.

Arterial BP and Renal Hemodynamics

MAP and renal function were measured in anesthetized (Inactin 110 mg/kg; RBI) rats, as previously described. Briefly, after induction of anesthesia, catheters were placed in left femoral artery (for blood sampling and blood pressure measurement), left femoral vein (for infusion of fluids), jugular vein (for infusion of H-inulin), and bladder (for urine collections). Renal blood flow was measured by determining extraction of H-inulin across the kidney, using a needle (23 gauge) attached to PE-50 and inserted in the retrograde position in the renal vein. All clearance experiments were timed exactly so that the blood samples for PRA were taken at the same time after induction of anesthesia in each rat, ie, when the clearance studies were completed (135 minutes after induction of anesthesia). The kidneys were perfused free of blood with PBS containing 2% heparin, removed, and weighed.

Urinary Protein Excretion

Urine was collected for 24 hours, and protein excretion was measured as we have previously described.

Plasma Renin Activity

PRA was measured by radioimmunoassay, as previously described.

Serum Estradiol and Testosterone

Estradiol and testosterone were measured by radioimmunoassay (ultra-sensitive estradiol kit, Diagnostic Systems Laboratories; Coat-A-Count Total Testosterone, Diagnostic Products Corp).

Urinary or Plasma F2-Isoprostanes

F2-isoprostanes were measured in urine and plasma by gas chromatography and mass spectrometry, as previously described.

Statistical Analyses

Statistical differences for all data were determined by ANOVA or Student t test, by using Statview 512 and Dunnett test. Data are expressed as mean±SEM.

Results

Effect of Age on Sex Hormones and Vaginal Smears

As shown in Figure 1, plasma estradiol was measured during the reproductive cycle of young female rats. Estradiol levels were highest in females in diestrous stage and lowest in proestrous stage. Aging intact females stopped cycling at 10 to 12 months of age, as defined by 8 weeks of continuous estrous-looking cells. Chronic ovx resulted in atrophy of the vagina, as determined by reductions in weight of the tissue (data not shown), and vaginal smears revealed mucus with few cells. After ovx at 8 months, serum estradiol in old ovx rats at 18 months of age was lower than in females in proestrous. Estradiol levels in intact females, age 18 months, were similar to those in young females in proestrous and in old male SHR, but were higher than in old ovx rats.

MAP in Pre- and Postmenopausal SHR

Figure 2 represents the MAPs for premenopausal SHR, PMRs, and ovx rats. BP is also included for males for comparison. At 4 months of age, males have BP that is 25 to 30 mm Hg higher than that of females. Ovariectomy in females, at 4 to 7 weeks of age, had no effect on BP when they were 4 months old. At 8 months of age, the BP in intact females was not different from that in females, 4 months of age, but was still lower than that in age-matched males. Thus, the sex difference in BP is still present at 8 months of age. In 18-month-old ovx rats, there was no change in BP compared with that of premenopausal females, 8 months of age. In contrast, BP in PMRs (intact females, age 18 months) was ≈10% higher than that in old ovx rats. By 18 months of age, the gender difference in BP was no longer present, and BP was not different between PMRs and males. BP did not change significantly in male SHR from 8 to 18 months. Therefore, the loss of the gender difference in BP was not due...
to a reduction in BP with age in males but rather to an increase in BP in aging females.

Renal Hemodynamics in PMRs

Body weights were 20% or 50% higher in old ovx rats than in PMRs or premenopausal rats (4 months), respectively (Table). Kidney weights were similar between ovx rats and PMRs but were 20% higher than in premenopausal females (Table). As shown in Figure 3A, glomerular filtration rate (GFR) was similar in old ovx rats and PMRs, and GFR in both was lower than in young females. Renal plasma flow (Figure 3B) was similar in ovx rats compared with young females (P=0.08), but was significantly decreased in PMRs compared with young (30% decrease) or old ovx (50% decrease) rats. Because of the increased BP and reduced renal plasma flow in PMRs, renal vascular resistance (Figure 3C) was 80% higher in PMRs than in young or old ovx rats.

Proteinuria in ovx Rats and PMRs

As an index of renal injury, urinary protein excretion was measured. Old rats excreted significantly more protein than did young rats (Table). Surprisingly, old ovx rats excreted more protein than did PMRs.

Mechanisms That Could Contribute to Postmenopausal Hypertension

Various measurements of factors that are known to be changed after menopause in women and that could impact the BP in PMRs were measured as described below.

**Serum Testosterone**

Serum testosterone has been shown to be increased in some, but not all, studies of PMW. In the present study, serum testosterone was increased by 60% in old ovx rats compared with premenopausal females (Table). Testosterone was increased by 400% in PMRs compared with young females and by 200% compared with old ovx rats.

**Plasma Renin Activity**

PRA has been shown to be increased in PMW. In the present study, PRA was increased by 50% in PMRs compared with young females (Table). PRA in old ovx rats was not increased compared with young females.

**Effect of Antioxidants on BP and Renal Function**

PMW have been shown to have elevated levels of plasma F2-isoprostanes, an indicator of oxidative stress. Plasma F2-isoprostanes were also increased in PMRs compared with young females (Table). Therefore, because F2-isoprostanes and PRA were increased in PMRs and many studies have shown that Ang II can stimulate oxidative stress, we determined whether chronic antioxidant treatment (8 months) would affect BP in old males and PMRs. PMRs and male SHR were treated with vitamins E and C, whereas in males, tempol, but not vitamins E and C, reduced BP.

**Discussion**

There is a sex difference in BP in SHR with males typically having higher BP than that of females. In the present study, we present data showing that the BP increases in aging intact female SHR, after cessation of cycling, to the point at which the sex difference in hypertension disappears. This is consistent with epidemiological data in women. In the Third National Health and Nutrition Examination Survey (NHANES III) study, the investigators found that the prevalence of hypertension increased in women after the age of menopause (average, 51.4 years) until women had a higher prevalence of hypertension than did age-matched men. To

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**Characteristics of Aging in Female SHR**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight g</th>
<th>Kidney Weight g</th>
<th>U,V mg/24 h</th>
<th>F2-Isop ng/mL</th>
<th>PRA ng/Al/mL per hour</th>
<th>Testosterone ng/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mos (n=8)</td>
<td>197.3±3.9</td>
<td>1.66±0.03</td>
<td>1.23±0.23</td>
<td>0.55±0.07</td>
<td>10.75±1.69</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Old ovx (n=9)</td>
<td>293.7±8.7</td>
<td>2.05±0.06*</td>
<td>3.05±0.27*</td>
<td>ND</td>
<td>11.20±1.70</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>PMR (n=9)</td>
<td>240.9±4.6†</td>
<td>2.03±0.04*</td>
<td>2.12±0.23†</td>
<td>57.6±3.8†</td>
<td>16.61±2.23†</td>
<td>3.7±0.4†</td>
</tr>
</tbody>
</table>

ND indicates not determined; old ovx, female SHR that were ovariectomized at 8 months and aged to 18 months; PMR, postmenopausal rats, aged 18 mos; U,V, urinary protein excretion; F2-Isop, plasma F2-isoprostanes; and PRA, plasma renin activity; testosterone, serum testosterone.

*P<0.05 vs 4-month females; †P<0.05 vs old ovx.
our knowledge, ours is the first study to describe significant increases in BP in postcycling female rats.

To have an appropriate and useful animal model of postmenopausal hypertension, we have identified several characteristics found in PMW that we believe are important in the model. For example, estradiol must be reduced, and the animal must have ceased to cycle.21 This could be accomplished by normal aging or by ovx. However, the animal should also be aged in order to reproduce the aging found in PMW. Ideally, PRA should be increased and oxidative stress should be present, as previously found in PMW.2,3,17 We believe we have met these requirements in our aged intact female SHR.

One possible criticism of our model is that it may be difficult to dissect mechanisms responsible for an increase in BP with age in an animal that was already hypertensive as a young adult.15 To address this concern, in this model, we have the opportunity to use either ovx females or males as controls because the SHR is a genetic model of essential hypertension. For example, in the present study, we found that the ovx rats did not exhibit increases in BP, whereas the PMRs did. The rats in both groups were the same age. Thus, aging alone did not contribute to the increased BP in the PMRs. Similarly, body weight was higher in ovx rats than in PMRs. Therefore, increased body weight did not play a role in the higher BP in PMRs.

As mentioned above, menopause in women is characterized by reductions in estradiol. PMRs had similar estradiol levels as found in aged male SHR or young females in proestrous. In contrast, serum testosterone was increased by 400% in PMRs compared with young females. In women, whether testosterone changes after menopause is controversial. Independent studies in PMW have found serum testosterone increases with age, decreases with age, or remains unchanged.16,21 Further studies in PMW will be necessary to define if androgens change or not. Perhaps the absolute values are not as important as the ratio of estrogens to androgens when trying to elucidate mechanisms responsible for cardiovascular disease and hypertension in PMW. It should be mentioned that despite the increase in testosterone in PMRs, male SHR, whether young or old, have serum testosterone levels that are significantly higher than levels in PMRs.15 So whether the relative increase in androgens plays a role in the increased BP of PMRs is not clear from the present study.

It is also not clear why BP did not increase in old ovx rats. BP does increase with age in women who have undergone

**Figure 3.** Renal hemodynamics in female SHR: young females, 4 months of age (n = 8); ovx rats (n = 8); and PMRs (n = 8), aged 18 months. A, GFR in female SHR. B, Renal plasma flow in female SHR. C, Renal vascular resistance in female SHR. *P < 0.05 vs young females; ‡P < 0.05 vs ovx rats.

**Figure 4.** Urinary F2-isoprostane excretion (A) and MAP (B) in male SHR and PMRs, aged 16 months, in control or in response to 8 months treatment with vitamins E and C or tempol (n = 6 to 7/group), as described in Methods. *P < 0.05 vs control PMRs; ‡P < 0.05 vs old control males.
oophorectomy. The PRA was similar in both young female and old ovx SHR, whereas PRA was significantly increased in PMRs. Thus, this difference in PRA is a plausible mechanism by which BP could be lower in old ovx rats than in PMRs. It is also possible that the small increase in serum testosterone in old ovx rats (60%) was not sufficient to impact BP, whereas the 4-fold increase in PMRs may have been. Androgens have been shown to have a stimulatory effect on renal angiotensinogen expression and, thus, could cause an increase in PRA, which is supported by our data. Our data also suggest that the source of the higher androgens in PMRs may be the ovary, because in the absence of the ovaries (old ovx rats), serum testosterone was 50% lower than in PMRs. Burger found that the ovary in the PMW is also an androgen-secreting organ. Thus, removal of the ovaries from our rats before cessation of cycling tended to cause protection from the increase in BP found in PMR. In general, with the exception of age, cessation in cycling and reduction in estradiol, the aged ovx female SHR does not meet the criteria we have set for a useful model of postmenopausal hypertension, whereas the PMR does.

Finally, to begin to dissect possible mechanisms by which BP increases in PMRs, we investigated the role that oxidative stress may play. Studies have shown that oxidative stress is increased in PMW. Also, the length of time after menopause has been positively correlated with increasing oxidative stress, as measured by plasma malondialdehyde. In the present study, we also found that plasma F₂-isoprostanes were elevated in the PMRs compared with young females, but was similar in old males, suggesting that oxidative stress was higher in PMRs than young females, and that the level of oxidative stress was similar in PMRs and old males. However, PMRs excreted 200% more F₂-isoprostanes than did males, suggesting differences in renal handling of F₂-isoprostanes between PMRs and old males.

Treatment of PMRs with vitamins E and C caused a 16 mm Hg reduction in BP, but had no effect in males. In males only tempol reduced BP (by 27 mm Hg). F₂-isoprostane excretion also supported these findings, showing lipid peroxidation was reduced in PMRs with vitamins E and C and in males with tempol. However, F₂-isoprostanes were reduced in PMRs and old males with tempol and vitamins E and C, respectively. Obviously, these reductions in oxidative stress were insufficient to have an impact on BP. Other investigators have shown that vitamin C alone reduces BP in young male SHR; however, they did not measure indexes of oxidative stress. In these studies, the doses of vitamin C were much higher than in the present study. Furthermore, the reductions in BP in response to the antioxidants are not correlated with changes in excretion rate of F₂-isoprostanes. Tempol reduced BP by 27 mm Hg in old males but only resulted in a 50% reduction in isoprostanes. In females, vitamins E and C produced a 16 mm Hg reduction in BP but resulted in a 70% reduction in isoprostanes. Taken together, the data suggest that the mechanisms producing oxidative stress, and thus impacting BP, may be different in old male and female SHR. tempol is a superoxide dismutase mimetic and vitamins E and C are generic antioxidants that interact with superoxide, peroxides, and hydroxyl radicals.

Perspectives
Why BP increases in PMW has not been defined. Loss of estrogen after menopause has been presumed to be the cause. However, hormone replacement therapy does not consistently reduce BP or protect against primary or secondary cardiovascular disease. The time course of the increase in BP is also not consistent with reduction in estrogens, because it does not occur as soon as the ovaries stop functioning but occurs gradually over years. This suggests that other secondary mechanisms may play a role in mediating the hypertension. We hope to use aged PMRs as a model for postmenopausal hypertension and to elucidate some of the mechanisms responsible. For example, despite the very low levels of serum testosterone in PMRs compared with males, we plan to determine if the relative increase in androgens in PMRs, compared with young females, plays a role in the increased BP. The balance between estrogens and androgens may be of more importance to the development of postmenopausal hypertension than either sex steroid alone. The role of the renin-angiotensin system in postmenopausal hypertension must also be determined because PRA is increased in PMW and PMRs. Finally, there are many ways in which oxidative stress can impact BP, such as an increase in vasoconstrictor substances (eg, endothelin, F₂-isoprostanes, and thromboxane A₂) and/or a decrease in vasodilator substances (eg, prostacyclin). These possible mechanisms will be evaluated in future studies.

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