Abolition of End-Organ Damage by Antiandrogen Treatment in Female Hypertensive Transgenic Rats

Ovidiu Baltatu, Cécile Cayla, Radu Iliescu, Dmitrii Andreev, Michael Bader

Abstract—We aimed at studying the role of androgens in the development of cardiovascular pathology in hypertensive female rats. Female TGR(mREN2)27 rats harboring the mouse Ren-2 renin gene were treated with Flutamide (specific antagonist of the androgen receptor, 30 mg/kg per day) starting at 4 weeks of age. Flutamide treatment significantly attenuated the development of hypertension in female rats (systolic blood pressure: treated, 134.5 ± 5.4 versus control, 165.4 ± 3.8 mm Hg). Heart hypertrophy was significantly reduced by the treatment (treated, 0.37 ± 0.008 versus control, 0.45 ± 0.01 g/100 g body wt). Urinary albumin excretion was blunted (treated, 0.4 ± 0.1 versus control, 23.1 ± 7.5 mg/24 hours), collagen III mRNA was significantly decreased, and no histological characteristics of end-organ damage were observed in the kidney after treatment. Flutamide treatment significantly reduced plasma renin concentrations and rat renin mRNA in kidney but not plasma angiotensinogen levels. Plasma levels of estrogens, testosterone, and luteinizing hormone were not altered. These results demonstrate that the androgen receptor antagonist Flutamide protects against hypertension and end-organ damage not only in male but also in female TGR(mREN2)27 rats. (Hypertension. 2003; 41[part 2]:830-833.)

Key Words: hypertension, renal ■ rats, transgenic ■ receptors, androgen ■ antihypertensive agents ■ albuminuria

H uman and laboratory studies have demonstrated a sexual dimorphism in hypertension.1 Gender differences have been observed in various hypertensive animal models, with male rats having higher blood pressure than female rats,2–6 including the transgenic rat TGR(mREN2)27 with an overactive renin-angiotensin system (RAS).7,8 Evidence is accumulating that androgens may play an important role in gender-associated differences in cardiovascular pathology. We have previously demonstrated that androgens contribute not only to the development of malignant hypertension but also to the associated end-organ damage in transgenic male TGR(mREN2)27 rats with overactive RAS.9 Females produce androgens as well, which act on specific receptors.10 Women with hyperandrogenism are considered to be at increased risk for cardiovascular disease.11–15 Based on a prospective literature survey, it has been recently hypothesized that relative androgen excess is a key factor explaining the increased risk of cardiovascular disease in interpubal women.16 In the present study, we hypothesized that endogenous androgens may participate in the development of hypertension and end-organ damage in female TGR(mREN2)27 rats. Androgen blockade was achieved by treatment with the antagonist Flutamide, and blood pressure (BP) as well as end-organ damage, RAS, and sex hormones were evaluated.

Methods

Rat Strains

Female transgenic heterozygous rats [TGR(mREN2)27] (n=24 rats) were obtained from the animal facilities of the Max-Delbrück-Center for Molecular Medicine, Berlin, Germany. The rats were housed individually, synchronized to a 12-hour light-dark cycle, at ambient temperature 23±2°C. A standard rat diet (ssniff R-ZUCHT) and tap water were supplied at libitum.

Study Design

All experimental protocols were performed in accordance with the guidelines for the use of laboratory animals by the Max-Delbrück-Center for Molecular Medicine and approved by an ethics committee.

To study the involvement of androgens in the development of hypertension and end-organ damage in female TGR(mREN2)27 rats, we treated them with Flutamide (specific nonsteroidal competitive antagonist of the androgen receptor, 30 mg/kg per day subcutaneously17 starting at 4 weeks of age (n=12) before the development of hypertension. A group of 12 rats received subcutaneous injections solely of the Flutamide solvent and represented the reference group. BP development was followed telemetrically, as previously described.18

At the age of 12 weeks, when the hypertension levels became stable, 24-hour urine was collected and the rats were killed by decapitation under light ether anesthesia. Plasma was collected for hormone analysis. Cardiovascular organs were excised for histology and gene expression analysis.
Kidney Damage Evaluation
Urine was collected by placing the rats into metabolic cages for 24 hours. Rat urinary albumin (index of kidney damage) was determined by ImmunoMannics (Bensheim, Germany) with a specific ELISA. To evaluate kidney fibrosis, collagen III mRNA was determined by ribonuclease protection assay. For histological analysis, kidney was excised, decapsulated, and fixed with 10% formalin in 0.01 mol/L PBS, pH 7.4, and dehydrated by immersing them stepwise into various concentrations of ethyl alcohol from low to high. The tissues were then embedded in paraffin and sectioned into 4-μm-thick slices, and the sections were stained with Goldner trichrome.9 Cytoplasm, muscle tissue, and erythrocytes stain red; collagen stains green. At least 5 randomly selected areas per sample were observed.

Heart Hypertrophy Evaluation
The hearts were excised, washed in ice-cold saline, blotted dry, and weighed. The left ventricles were separated and weighed.

Hormone Measurements
Plasma was obtained from trunk blood collected on EDTA (6.25 mmol/L) after centrifugation at 4000 rpm. Plasma renin concentration and activity were determined with an indirect enzyme-kinetic assay based on the generation of angiotensin I with modification of the pH optimum to measure rat and mouse plasma renin activity (PRA) and plasma renin concentration (PRC), based on a published report.10 Angiotensinogen, testosterone, estrogens, and luteinizing and follicle-stimulating hormones were measured by radioimmunoassay.

Gene Expression Studies
Total RNA was isolated from the kidney with the TRIzol Reagent (Life Technologies), followed by chloroform-isopropanol extraction, according to the protocol of the manufacturer. Specific mRNAs for rat or mouse renin or collagen III (marker of fibrosis) were determined by ribonuclease protection assay (RPA), with the use of the Ambion RPA II kit (AMS Biotechnology), as described previously.20 For semiquantitative determination of mRNA levels, band intensities were normalized to the housekeeping gene β-actin.

Statistical Analysis
Data were analyzed by independent-samples t test between 2-group comparisons or by GLM (general linear model) – general factorial or repeated-measures procedure (software SPSS 8.0) for multigroup and multifactorial analysis. Criterion for significant differences between groups of study was a probability value <0.05. Results are expressed as mean ± SEM.

Results
Effect of Flutamide Treatment on Body Weight
The body weight was not significantly altered by the Flutamide treatment (Flutamide, 274.2 ± 3.7 g versus control, 256.7 ± 8.8 g at the end of experiment).

Effect of Flutamide Treatment on Renin-Angiotensin System and Sex Hormones
Plasma renin concentrations and activities were drastically decreased after Flutamide treatment (Table 1). RPAs for rat or mouse renin and also the onion-shaped proliferative lesions (kidney morphology) disappeared after Flutamide treatment (Figure 4). The cardiac and left ventricular hypertrophies were reduced by Flutamide treatment (Figure 5).

Effect of Flutamide Treatment on Blood Pressure
Untreated TGR(mREN2)27 rats had fulminant hypertension starting at 10 weeks of age, as measured by telemetry (Figure 1). Flutamide decreased significantly the levels of systolic BP (Figure 1). At 12 weeks of age, the BP was significantly decreased in Flutamide-treated rats in comparison with control group, without alterations of heart rate (systolic BP, 134.5 ± 5.4 versus 165.4 ± 3.8 mm Hg; mean BP, 114.4 ± 4.8 versus 143.1 ± 3.9 mm Hg; diastolic BP, 95.3 ± 4.0 versus 120.5 ± 3.9 mm Hg; heart rate, 362.2 ± 4.2 versus 362.6 ± 4.7 beats/min, respectively).

Effect of Flutamide Treatment on End-Organ Damage
Untreated hypertensive TGR(mREN2)27 rats had signs of malignant hypertension with end-organ damage, including renal and cardiac pathology. The urinary albumin (as index of kidney damage) was drastically reduced by Flutamide treatment (Figure 2). Furthermore, the collagen III mRNA levels, as a marker of fibrosis, were significantly decreased in kidney (Figure 3). In agreement, the fibrinoid necrosis of arterioles and also the onion-shaped proliferative lesions (kidney morphology) disappeared after Flutamide treatment (Figure 4). The cardiac and left ventricular hypertrophies were reduced by Flutamide treatment (Figure 5).

Figure 1. Effect of androgen receptor antagonism on the development of hypertension in female TGR(mREN2)27 rats, untreated group (control, n = 6) compared with group treated with Flutamide (n = 6). **Significantly different (P < 0.01).

Figure 2. Effect of androgen receptor antagonism on urinary albumin excretion in female TGR(mREN2)27 rats, untreated group (control, n = 8) compared with group treated with Flutamide (n = 7). ***Significantly different (P < 0.001).

Figure 3. Effect of androgen receptor antagonism on kidney collagen III mRNA in female TGR(mREN2)27 rats, untreated group (control, n = 4) compared with group treated with Flutamide (n = 4). *Significantly different (P < 0.05).
mouse renin mRNAs in kidney revealed significantly decreased levels after Flutamide treatment (Table 1). Plasma angiotensinogen as well as plasma estrogens and testosterone levels were not altered by Flutamide treatment (Tables 1 and 2).

**Discussion**

The major findings of this study are (1) Endogenous androgens contribute to the development of malignant hypertension and end-organ damage induced by an overactive RAS not only in male rats but also in female rats. (2) The antiandrogen Flutamide prevents hypertension in female rats probably as the result of a marked inhibition of the RAS, by decreasing the renin synthesis and activity.

Several reports have indicated a role of androgens in hypertensive male subjects (reviewed in Reference 21). Androgens are produced by female subjects as well, and like estrogens may have cardiovascular implications. This is why we aimed at studying the relevance of endogenous androgens in the development of cardiovascular pathology in female rats. We investigated the consequences of endogenous androgen blockade by Flutamide for hypertension and end-organ damage in female TGR(mREN2)27 rats. The TGR(mREN2)27 rats represent a model of fulminant hypertension with a defined genetic cause, an overactive RAS. The results presented in this study demonstrate that endogenous androgens contribute to the development of malignant hypertension induced by an overactive RAS not only in male subjects but also in female subjects. Moreover, androgen blockade by Flutamide in female TGR(mREN2)27 rats not only significantly attenuated the development of hypertension but also totally prevented kidney damage, as evidenced by the absence of albuminuria, reduction of collagen III mRNA levels and the normal histological picture. Furthermore, a significant decrease of cardiac and left ventricular hypertrophy was observed.

Data from experimental animals, epidemiological surveys, and clinical investigations suggested effects of sex hormones and gender on RAS at several levels and still remain subject of investigations. We and others have demonstrated that renin gene expression and activity can be regulated by androgens in males. Indeed, in the present study, blockade of androgen receptors with Flutamide in females induced a drastic decrease of plasma renin concentrations and activity, at least to a similar degree as in males. Moreover, the kidney mRNA levels for rat and mouse renin were reduced, in correlation to lower plasma concentrations and activities. However, plasma levels of angiotensinogen were not altered, suggesting that endogenous androgens do not alter its production, at least in this experimental model. Previous studies indicated that estrogens downregulate renin, ACE, and AT-1 receptors, while upregulating angiotensinogen (reviewed in Reference 25). Therefore, Flutamide might decrease renin production and activity as the result of unopposed estrogen actions after the blockade of androgen actions.

<table>
<thead>
<tr>
<th>TABLE 1. Effect of Androgen Receptor Antagonism on PRC and PRA, Angiotensinogen, and Kidney Renin mRNA Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAS Components</strong></td>
</tr>
<tr>
<td>Rat renin mRNA, % of β-actin mRNA</td>
</tr>
<tr>
<td><strong>Control</strong> (n=7)</td>
</tr>
<tr>
<td>Rat PRC, ng AngI/mL per hour</td>
</tr>
<tr>
<td>41.3±4.9</td>
</tr>
<tr>
<td>Rat PRA, ng AngI/mL per hour</td>
</tr>
<tr>
<td>11.8±0.9</td>
</tr>
<tr>
<td>Plasma angiotensinogen, μg/mL</td>
</tr>
<tr>
<td>1.1±0.08</td>
</tr>
<tr>
<td><strong>Flutamide</strong> (n=6)</td>
</tr>
<tr>
<td>Rat renin mRNA, % of β-actin mRNA</td>
</tr>
<tr>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Mouse renin mRNA, % of β-actin mRNA</td>
</tr>
<tr>
<td>0.27±0.06</td>
</tr>
<tr>
<td>Mouse PRA, ng AngI/mL per hour</td>
</tr>
<tr>
<td>11.4±1.4</td>
</tr>
<tr>
<td>Plasma angiotensinogen, μg/mL</td>
</tr>
<tr>
<td>6.6±0.6</td>
</tr>
<tr>
<td><strong>Control</strong> (n=7)</td>
</tr>
<tr>
<td>1.0±0.1</td>
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<tr>
<td><strong>Flutamide</strong> (n=6)</td>
</tr>
<tr>
<td>0.1±0.02</td>
</tr>
<tr>
<td><strong>P&lt;0.05, †P&lt;0.001, significantly different Flutamide group vs control group.</strong></td>
</tr>
</tbody>
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**Figure 4.** Abolition of hypertension-induced kidney histopathology by Flutamide treatment in female rats. Fibrinoid necrosis, concentric wall thickening of arterioles, and hyperplastic arteriolosclerosis is observed in the kidney of untreated rats (A). Flutamide-treated female TGR(mREN2)27 have a completely normal histological aspect of the kidney (B).

**Figure 5.** Effect of androgen receptor antagonism on heart and left ventricular (LV) hypertrophy in female TGR(mREN2)27 rats, untreated group (control, n=12) compared with group treated with Flutamide (n=12). ***Significantly different (P<0.001).
Flutamide is an androgen receptor antagonist used in the treatment of advanced prostate cancer\textsuperscript{28} or polycystic ovary syndrome.\textsuperscript{29} On the opposite of males, Flutamide treatment does not induce a feedback increase of testosterone\textsuperscript{29} or estrogens\textsuperscript{30} in females. Indeed, in our study, Flutamide did not alter plasma testosterone or estrogen levels. This excludes the possibility of any cardiovascular effect caused by increased levels of testosterone or estrogens, for example, nongenomic vascular actions.\textsuperscript{24–34} However, we cannot exclude that Flutamide itself may exert similar direct cardiovascular actions independent of its antiandrogens properties and therefore equally effective in both sexes. This would explain the fact that Flutamide can induce comparable antihypertensive and renin-lowering effects in both males and females. In support of this, it is known that Flutamide has no agonistic actions on the nuclear androgen receptors.\textsuperscript{28} However, this hypothesis needs further investigations.

**Acknowledgments**

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**References**

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