Pressor Mechanisms in Adriamycin-Induced Nephropathy With Hypertension in Rats

Roberto Franco, Ana Gut, Aparecida Ferrari-Spadotto, Jose Georgette, Irene Gavras, Haralambos Gavras

Abstract We explored the role of angiotensin II and vasopressin in the maintenance of blood pressure during the nephrotic syndrome of adriamycin-induced nephropathy in rats. All 91 rats treated with adriamycin developed chronic renal failure with nephrotic syndrome, which was more pronounced in the normotensive rats than the 35% who became hypertensive. Angiotensin II blockade with DuP 753 produced a significantly greater hypotensive response in both the adriamycin-hypertensive (-16±3 mm Hg) and adriamycin-normotensive (-14±5 mm Hg) groups than the saline-treated controls (-5±1 mm Hg, P<.05). Vasopressin blockade with either a V₁V₂ inhibitor or a selective V₁ inhibitor produced a pronounced hypotensive response in adriamycin-hypertensive rats only (by -16±4 and -17±2 mm Hg, respectively, P<.01), although the nonselective vasopressin inhibitor produced similar fluid loss and body weight reduction in all three groups. The data suggest that in adriamycin-induced nephropathy with nephrotic syndrome, angiotensin II contributes to blood pressure maintenance in both hypertensive and normotensive animals, whereas the pressor action of vasopressin contributes to elevated blood pressure in hypertensive animals only. (Hypertension. 1994;23 [suppl 1]:I-246-I-249.)

Key Words • doxorubicin • receptors, vasopressin • angiotensin II • kidney failure

Hypertension frequently coexists with chronic renal failure, with a prevalence reported to vary between 23% and 88%.[6-12] Moreover, coexistence of hypertension with this condition appears to be a sign of poor prognosis.[2] It is usually unrelated to the degree of renal insufficiency[3] or to the presence or absence of nephrotic syndrome or steroid therapy, although remission of this syndrome can be associated with a decrease in blood pressure,[4] and coexistence of the two conditions tends to accelerate the progression of renal insufficiency.[5] The mechanism or mechanisms of high blood pressure maintenance in this setting are still not clearly understood and are believed to involve activation of several neurohumoral vasoactive systems, including the sympathoadrenal system, the renin-angiotensin-aldosterone axis, and vasopressin.

Adriamycin is an antineoplastic drug that in rats produces a nephrotic syndrome with mild chronic renal insufficiency within 8 to 10 weeks.[6-8] We used this model to explore the contribution of two vasopressor hormones—angiotensin II (Ang II) and vasopressin—to the hypertension associated with the nephrotic syndrome.

Methods Experiments were performed on adult, male Wistar rats with initial weights of 250 to 350 g. Adriamycin (2 mg/kg) was administered intravenously from the tail vein to 91 rats twice at 3 weeks apart. All animals had free access to tap water and standard rat chow, were handled humanely, and were killed with an intracardiac injection of sodium pentobarbital at the end of the experiment. The rats were studied 8 to 10 weeks after the first injection of adriamycin, when nephropathy with hypertension or normotension developed. Hypertension was defined as baseline mean arterial pressure (MAP) above 125 mm Hg in nonanesthetized rats. The day before the experiment the rats had catheters inserted into the right femoral artery and jugular vein (PE-50, Intramedic, Becton Dickinson, Parsippany, NJ) under light ether anesthesia. Blood pressure and heart rate (HR) were monitored through a Gould Statham P23ID pressure transducer (Gould Inc, Cleveland, Ohio) connected to the femoral catheter and were recorded on a Gould 3200 paper chart recorder.

On the day of the experiment, the animals were conscious and unrestrained in plastic cages, with continuous monitoring of blood pressure and HR. A 1- to 2-hour stabilization period was observed before initiation of the experiments. Forty-nine adriamycin-injected rats were included in the study, allocated to one of two groups: hypertensive (ADRH, n=25) or normotensive (ADRIN, n=24). A control group (S, n=24) was composed of normal rats that received 0.9% saline instead of adriamycin. To examine the role of Ang II and vasopressin in this nephropathy model, nine subgroups were studied: Three ADRH groups submitted to either Ang II blockade with DuP 753 (ADRH+DuP, n=8) or vasopressin blockade with a V₁V₂ antagonist (ADRH+V₁V₂, n=9) or a V₁ antagonist (ADRH+V₁, n=7); three ADRIN groups (ADRN+DuP, n=8; ADRN+V₁V₂, n=9; and ADRN+V₁, n=7); and three control groups (S+DuP, n=8; S+V₁V₂, n=9; and S+V₁, n=7). DuP 753 was given at a dose of 6 mg/kg IV. MAP and HR were followed for 1 hour before and 3 hours after DuP 753. The V₁ antagonist was given at a dose of 120 μg/kg, and the same procedures as above were carried out except for the weight measurement. At the end of each experiment, blood samples were collected for measurement of creatinine, albumin, and cholesterol levels. Three additional adriamycin groups—hypertensive (n=8), normotensive (n=8), and intact controls (n=8)—were used only for measurement of tail blood pressure and
collection of blood for measurement of plasma osmolality, urea, and electrolytes.

The DuP 753 was dissolved in 5% dextrose in water to a concentration of 6 mg/mL and the two vasopressin antagonists to 120 μg/mL. The vasopressin antagonists used were the analogues 1β-mercapto-β,β-cyclopentamethylenepropionic acid)-ω-Tyr(0-ethyl)-4-valine-arginine vasopressin as V1 antagonist and 1β-mercapto-β,β-cyclopentamethylenepropionic acid)-ω-(0-methyl)tyrosine-arginine vasopressin as V2 antagonist. Proteinuria was measured by a biuret method; creatinine, albumin, and cholesterol by a colorimetric-enzymatic method; and electrolytes by flame photometry. Plasma osmolality was measured with a freezing point osmometer (u OSMETTE 5004, Precision Systems Inc, Sudbury, Mass).

Statistical analysis was performed by ANOVA; differences were corrected by Bonferroni’s test for multiple comparisons, Tukey’s test for comparison of values within groups, and Duncan’s test for comparison of means of different groups. Data are expressed as mean±SEM.

### Results

The incidence of arterial hypertension in the 91 rats at 8 to 10 weeks after adriamycin was 35%. Baseline intra-arterial MAP after observation for 1 hour was 142±8 mm Hg in the ADRH group, 117±9 mm Hg in the ADRN group, and 107±7 mm Hg in the saline controls (P<.001 between the hypertensive and two normotensive groups). Proteinuria in the ADRN group, and 107±7 mm Hg in the saline controls (P<.001 between the hypertensive and two normotensive groups). The ADRH+V1V2 group had no significant change in MAP during blockade. However, both hypertensive and normotensive rats had a significant decrease in MAP at 90 minutes, with a nadir value at 210 minutes. HR showed no significant variation within or between groups.

In the three additional groups of rats in which only plasma osmolality and tail systolic blood pressure (TBP) were measured, there was a significant elevation of osmolality (P<.04) only in the hypertensive group (osmolality, 334±7 mOsm; TBP, 161±8 mm Hg) compared with the normotensive (osmolality, 317±12 mOsm; TBP, 140±9 mm Hg) or control (osmolality, 312±9 mOsm; TBP, 131±9 mm Hg) group. This was most likely in part due to higher blood urea levels in the hypertensive group (ADRH, 168±9 mg/dL, P<.001) compared with the normotensive (ADRN, 136±12 mg/dL) and control (S, 58±8 mg/dL) groups, whose values decrease at 180 minutes, with further lowering at 210 minutes sustained at 240 minutes. The only difference seen in HR was at 240 minutes, when the ADRH+V1V2 group was significantly higher than the ADRN+V1V2 group (373±18 versus 317±10 beats per minute). There was no difference in weight loss (as a measure of fluid balance) among groups after they received the V1V2 antagonist (ADRH, 21.1±2.9 g; ADRN, 21.4±2.4 g; S, 21.9±2.8 g).

Vasopressin blockade with the V1 antagonist also is shown in Fig 2 (bottom). The control (S+V1) group and ADRN+V1 group had no significant change in MAP during blockade. The ADRH+V1 group had a significant drop in MAP at 90 minutes, with a nadir value at 210 minutes.

### Blood Chemistry, Electrolytes, and Osmolality in Study Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ADRH</th>
<th>ADRN</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.8±0.9*</td>
<td>1.5±0.4*</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>1.5±0.11</td>
<td>1.6±0.21</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>303±177</td>
<td>352±261</td>
<td>85±41</td>
</tr>
<tr>
<td>Sodium, mEq/L</td>
<td>142±3</td>
<td>136±2</td>
<td>140±1</td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>4.6±0.3*</td>
<td>6.5±0.2*</td>
<td>5.4±0.1</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>168±9*</td>
<td>136±12*</td>
<td>58±8</td>
</tr>
<tr>
<td>Osmolality, mOsm/kg H2O</td>
<td>334±75</td>
<td>317±12</td>
<td>312±9</td>
</tr>
</tbody>
</table>

ADRH indicates adriamycin-hypertensive rats; ADRN, adriamycin-normotensive rats; and S, saline-treated control rats.

*P<.01, †P<.001 vs S.
‡P<.001, ‡P<.04 vs ADRN and S.

![Graph showing changes in mean arterial pressure induced by angiotensin II blockade with DuP 753 in adriamycin-hypertensive (A, n=8), adriamycin-normotensive (E, n=8), and saline-treated control (C, n=8) animals. *P<.05 vs time zero; #P<.05 vs adriamycin-nomotensive and control.](http://hyper.ahajournals.org/Downloaded from)
Fig 2. Line graphs show changes in mean arterial pressure induced by a nonselective V1V2vasopressin antagonist (top) and selective V1 vasopressin antagonist (bottom) in adriamycin-hypertensive (V, V2, n=9; V1, n=7), adriamycin-normotensive (V, V2, n=9; V1, n=7), and saline-treated control (V, V2, n=8; V1, n=7) animals. *P<.05 vs time zero.

were significantly lower than the ADRN group (P<.001).

Fig 3 summarizes the results of Ang II and vasopressin blockade on arterial pressure in adriamycin rats and controls. DuP 753 lowered blood pressure to the same extent in both normotensive (−14±5 mm Hg) and hypertensive (−16±3 mm Hg) rats, significantly more than in controls (−5±1 mm Hg, P<.05). In contrast, the two vasopressin antagonists decreased MAP only in the hypertensive (V, V2, −16±4 mm Hg; V1, −17±2 mm Hg, P<.01) groups, without change in adriamycin normotensive (V, V2, −3±1 mm Hg; V1, −3±2 mm Hg) or control (V1V2, −2±2 mm Hg; V1, −4±2 mm Hg) groups.

Discussion

Experiments in the present study demonstrate that Ang II contributes to the maintenance of blood pressure at normal levels as well as to the genesis of hypertension in rats with nephropathy induced by adriamycin. In addition, vasopressin-mediated vasoconstriction is one component of the hypertension in this model. Vasopressin is probably activated in part by increased plasma osmolality as a consequence of elevated blood urea nitrogen levels and in part by the reduction of effective intravascular fluid volume characteristic of the nephrotic syndrome. The fact that both a V1 and V1V2 antagonist produced similar degrees of blood pressure lowering suggests that both agents produced their hypotensive effect via blockade of the V1 receptor. Indeed, weight loss, indicating V2-mediated negative fluid balance, was similar in hypertensive and normotensive rats; yet only the former exhibited a fall in blood pressure, suggesting that the fall was due to release of vasoconstriction rather than to further volume contraction.

As originally described by Okuda et al., arterial blood pressure in the adriamycin nephropathy model rises significantly at week 8. In our experiment hypertension developed in 35% of rats. A nephrotic syndrome characterized by proteinuria, hypoalbuminemia, and hypercholesterolemia, as described before, developed in all animals at 8 to 10 weeks and was accompanied by significantly elevated serum creatinine levels.

A common feature of edematous disorders, including the edema of nephrotic syndrome, is the mobilization of neurohumoral vasopressor mechanisms such as the sympathoadrenal system, the renin-angiotensin-aldosterone axis, and vasopressin. These mechanisms contribute both to preservation of intravascular effective blood volume and to abnormal elevation of arterial blood pressure and expansion of extravascular fluid volume. Elevated plasma renin activity and angiotensin-converting enzyme activity have been reported in rats with puromycin nephrotic syndrome. Acute or chronic treatment with angiotensin converting enzyme inhibitors lowered blood pressure in rats with puromycin nephropathy or experimental membranous nephropathy. Abnormal retention of sodium and fluid is also believed to participate in the pathogenesis of hypertension in these nephropathies, in which an interaction of sodium-dependent and renin-dependent vasoconstrictor mechanisms is occurring. Indeed, it is likely that there is a change in the prevailing mechanisms at different times during the course of the disease. The significant blood pressure fall in response to the type 1 angiotensin receptor subtype antagonist DuP 753 in both normotensive and hypertensive adriamycin rats in the present experiments indicates that Ang II is activated in this stage of adriamycin nephropathy regardless of actual blood pressure levels.

Vasopressin is also known to contribute to elevated blood pressure in advanced chronic renal failure. Increased osmolality due to retention of both sodium and blood urea may contribute to stimulation of vasopressin in this setting. In nephrotic syndrome with edema, vasopressin was found to be elevated before and during infusion of human serum albumin and to
decrease during head-out immersion in water,22 a maneuver known to increase effective central blood volume. In the present study a blood pressure--lowering effect was observed in the adriamycin hypertensive animals only, and it occurred in response to either a selective V₁ or a nonselective V₁V₂ vasopressin receptor antagonist. The body weight and fluid loss in response to the nonselective antagonist was similar for both the hypertensive and normotensive groups with adriamycin nephropathy, indicating that the mechanism of this antihypertensive effect was elimination of V₁-mediated vasoconstriction rather than fluid volume contraction.

In conclusion, our data suggest that in adriamycin-induced nephropathy with nephrotic syndrome, Ang II contributes to blood pressure maintenance in both hypertensive and normotensive animals, whereas the pressor function of vasopressin contributes to the elevated blood pressure of hypertensive animals only. It is unclear in these two groups with similar degrees of renal insufficiency and nephrotic edema at what point and why the interaction between vasoactive factors produces an abnormal elevation of blood pressure in some animals and not in others.

References

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