Effects of Omapatrilat on Blood Pressure and Renal Injury in L-NAME and L-NAME Plus DOCA-Treated Rats

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Background: This study investigates the effects of chronic administration of omapatrilat (OMA) on blood pressure (BP), renal injury, and other variables in N\textsuperscript{-}nitro-L-arginine methyl ester (L-NAME) hypertension and in the low-renin model produced by the simultaneous administration of L-NAME and deoxycorticosterone acetate (DOCA).

Methods: The control, DOCA, L-NAME, L-NAME/DOCA, L-NAME/OMA, and L-NAME/DOCA/OMA groups were used. Tail systolic BP was measured twice a week. After 4 weeks of treatment, mean arterial pressure (MAP), and metabolic, morphologic, and renal variables were measured.

Results: The final values of MAP were 109 ± 5.1 mm Hg for the control group, 113 ± 3.0 mm Hg for DOCA, 175 ± 3.7 mm Hg for L-NAME, 193 ± 3.8 mm Hg for L-NAME + DOCA, 117 ± 3.9 mm Hg for L-NAME + OMA, and 158 ± 3.0 mm Hg for L-NAME + DOCA + OMA. The rats treated with L-NAME showed mild and scarce renal lesions, which were prevented by OMA treatment and the L-NAME + DOCA group showed proteinuria and hyaline arteriopathy, which were markedly attenuated in the L-NAME + DOCA + OMA group. Plasma urea and creatinine were significantly increased in the L-NAME + DOCA group, whereas these variables were not significantly greater in the L-NAME + DOCA + OMA group versus controls. The L-NAME + DOCA group showed relative renal and cardiac hypertrophy that was not observed in the L-NAME + DOCA + OMA group.

Conclusions: The simultaneous blockade of neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) completely prevents L-NAME hypertension. Our results also show that OMA attenuates the increased BP and the renal injury in L-NAME hypertensive rats treated with DOCA. Assuming that this is a low-renin model of hypertension, the protective effect of OMA may be due to an increase in vasodilator peptides produced by both ACE and NEP inhibition. Am J Hypertens 2003;16:33–38 © 2003 American Journal of Hypertension, Ltd.

Key Words: L-NAME, DOCA, omapatrilat, hypertension, renal injury.

Angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) are cell membrane-bound zinc metallopeptidases, with close homology at their catalytic sites and several common substrates.\textsuperscript{1} Omapatrilat (OMA) is a mercaptoacyl derivative of a dipeptide surrogate that in vitro\textsuperscript{1} and in vivo\textsuperscript{2} inhibits simultaneously both ACE and NEP. Inhibition of NEP protects vasodilator peptides (natriuretic peptides, bradykinin, adrenomedullin) from degradation and lowers blood pressure (BP) in low renin states.\textsuperscript{3–5} The ACE inhibition attenuates the formation of angiotensin II (Ang II) and lowers BP in normal/high renin states.\textsuperscript{3,6}

Thus, OMA may be beneficial for treating several alterations in which Ang II is activated, such as the arterial hypertension secondary to nitric oxide synthesis (NOS) inhibition. The participation of the renin-angiotensin system (RAS) is supported by data showing that chronic blockade of RAS attenuates the development of L-NAME hypertension and the arteriolar and glomerular injuries produced in this model of hypertension.\textsuperscript{7–14} However, we...
previously reported that the chronic blockade of AT₁ receptors attenuated but did not normalize BP, indicating that other factors participate in the development of this hypertension model. Moreover, chronic administration of deoxycorticosterone acetate (DOCA) to L-NAME-treated rats increases BP, aggravates renal injury, suppresses plasma renin activity, and transforms the NO-deficient type of hypertension into an Ang II-independent model of hypertension, because chronic Ang II receptor blockade is ineffective to lower BP and ameliorate renal injury in these animals.

In the present study, we used OMA to take advantage of its dual mechanism of action, which could be more beneficial than only RAS inhibition or blockade to ameliorate the development of the arterial hypertension induced by L-NAME and in the low-renin model produced by the chronic administration of L-NAME and DOCA.

Methods

Animals

Male Wistar rats born and raised at the animal center of the University of Granada were used. The experiments were performed according to European Union guidelines for the ethical care of animals. Rats that initially weighed 200 to 250 g were randomly assigned to different experimental groups. All animals had free access to standard rat diet with a sodium content of 0.5% (rodent toxicology diet, B&K, Barcelona, Spain) and tap water ad libitum, except where stated. The rats were randomly divided into six groups: Control, L-NAME-treated, DOCA-treated (DOCA), L-NAME plus DOCA-treated (L-NAME + DOCA), L-NAME plus OMA-treated (L-NAME + OMA), and L-NAME plus OMA and DOCA-treated (L-NAME + DOCA + OMA) rats (n = 10 in each group). L-NAME was given by gavage (35 mg/kg per day), and OMA was given in the drinking water at a concentration of 40 mg/100 mL. DOCA was administered subcutaneously at a dose of 12.5 mg/rat per week. All treatments were started at the same time and were maintained for 4 weeks.

Experimental Protocol

Body weight and tail systolic BP (SBP) were determined twice a week during the course of the experiment. The SBP was measured by tail–cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain). At least seven determinations were made at every session, and the mean of the lowest three values within a range of 5 mm Hg was used to obtain the SBP level. After the time course study, all animals were housed in metabolic cages with free access to food and their respective drinking fluids. After 2 days of adaptation, the food and water intake and urine values were gathered during 2 consecutive days. The values obtained each experimental day were averaged for statistical purposes. The urinary variables measured were diuresis, natriuresis, kaliuresis, creatinine, and proteinuria.

After the metabolic study was completed, the femoral artery was cannulated and exteriorized at the dorsum of the neck. After a 24-h recovery period, direct BP and heart rate were recorded continuously for 60 min. The values obtained during each of the last 30 min were averaged to obtain the mean BP value. Blood samples from the femoral catheter were taken to determine plasma urea, creatinine, and electrolytes. Body weight, ventricular weight, and kidney weight were also measured at the end of the study.

Analytical Procedures

Sodium, potassium, urea, and creatinine were measured on the day by an autoanalyzer (Beckman CX4, Breca, CA). Proteinuria was measured by the method of Bradford.

Histologic Techniques

For conventional morphology, buffered 4% formaldehyde-fixed, paraffin-embedded longitudinal tissue sections in sagittal plane were stained with hematoxylin and eosin, Masson-Goldner trichromatic stain, and periodic acid-Schiff stain. The extent of vascular injury (proliferative arteriopathy, hyaline arteriopathy, and fibrinoid necrosis) was assessed by examining profiles of arteries and arterioles in a single kidney section. The presence of glomerular lesions (glomerulosclerosis, collapse, necrosis, and microaneurysm) was evaluated in at least 300 glomeruli. Tubular atrophy, tubular casts, and tubular dilation were also evaluated. The morphologic study was done in a blinded fashion on 3-μm sections with light microscopy, using the most appropriate stain for each lesion. The values were expressed as the percentage of rats with lesions in each group, and the severity of lesions was calculated semiquantitatively using a 0 to 3+ scale (−, absence; +, mild [<10% of vessel, tubules, or glomeruli involved]; ++, moderate [10% to 25%]; ++++, severe [>25%]).

Statistical Analyses

The evolution of tail SBP with time was compared using a nested design. When the overall difference was significant, Bonferroni’s method with an appropriate error was used. The other variables measured at the end of the experimental period were compared with one-way ANOVA, and subsequent pairwise comparisons were performed with the Newmann-Keuls test. The correlation analysis between the renal lesions, proteinuria and BP were performed using test of Spearman.

Results

Blood Pressure

Fig. 1 summarizes BP data. Fig. 1A depicts the evolution of the tail SBP measured by plethysmography and Fig. 1B shows the final MAP measured by direct recording in
conscious rats. The L-NAME treatment induced a time-dependent increase in tail SBP, which was significantly aggravated by the coadministration of DOCA. The tail SBP was higher in L-NAME + DOCA rats throughout the 4 weeks of the study. The administration of DOCA alone maintained BP at normal values. The simultaneous administration of OMA normalized BP in L-NAME rats and attenuated but did not normalize BP in L-NAME + DOCA-treated rats. The BP measurements from the femoral catheter in conscious rats at the end of the experiment confirmed the values obtained by the indirect method.

Morphologic Variables

Final body weight was similar in all of the experimental groups. No significant differences were found in left ventricular weight or kidney weight in the groups, except for the L-NAME + OMA group, which showed significant reduction in left ventricular weight versus controls. Relative left ventricular and kidney weight were significantly increased in the L-NAME + DOCA group, whereas in the L-NAME + DOCA + OMA group these variables were not significantly modified. In the L-NAME and DOCA groups, both these variables were similar to the controls, whereas in the L-NAME + OMA group relative left ventricular weight was significantly reduced (Table 1).

Histopathology Results

The renal lesions observed in L-NAME-treated experimental groups were mild and scarce, with only 20% of the rats (2 of 10) showing mild and scattered hyaline arteriopathy (Fig. 2). In the L-NAME + DOCA group, moderate hyaline arteriopathy (+ +; thickening of the vascular wall with narrowed lumen) was observed in 42.8% of this group and hyaline casts in 28.5%. No glomerular lesions (glomerulosclerosis, collapse, necrosis, microaneurysm) or major interstitial lesions (tubular atrophy, tubular dilation) were observed in any group. The presence of vascular lesion positively correlated with proteinuria (spearman test, \( P < 0.021 \)) and BP (s = 0.459; spearman test, \( P < 0.01 \)). OMA totally prevented renal lesion in L-NAME-treated rats and reduced the incidence and intensity of hyaline arteriopathy in L-NAME + DOCA-treated rats (11.1% ; 1 of 9; incidence at mild [+] intensity).

Table 1. Morphologic variables measured at the end of the experimental period

<table>
<thead>
<tr>
<th>Groups ((n = 10))</th>
<th>CONT</th>
<th>DOCA</th>
<th>NAME</th>
<th>NAME + DOCA</th>
<th>NAME + OMA</th>
<th>NAME + DOCA + OMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>377 ± 16</td>
<td>350 ± 9</td>
<td>344 ± 12</td>
<td>322 ± 5</td>
<td>330 ± 10</td>
<td>340 ± 6</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>643 ± 27</td>
<td>622 ± 19</td>
<td>593 ± 21</td>
<td>743 ± 28</td>
<td>413 ± 20*</td>
<td>619 ± 20</td>
</tr>
<tr>
<td>KW (mg)</td>
<td>882 ± 44</td>
<td>859 ± 40</td>
<td>803 ± 28</td>
<td>885 ± 33</td>
<td>817 ± 37</td>
<td>888 ± 29</td>
</tr>
<tr>
<td>L/VW/BW (mg/g)</td>
<td>1.71 ± 0.05</td>
<td>1.78 ± 0.04</td>
<td>1.74 ± 0.09</td>
<td>2.31 ± 0.10*</td>
<td>1.25 ± 0.04*</td>
<td>1.82 ± 0.05</td>
</tr>
<tr>
<td>KW/BW (mg/g)</td>
<td>2.35 ± 0.12</td>
<td>2.45 ± 0.08</td>
<td>2.34 ± 0.07</td>
<td>2.75 ± 0.10*</td>
<td>2.47 ± 0.08*</td>
<td>2.61 ± 0.07</td>
</tr>
</tbody>
</table>

CONT = control; DOCA = DOCA-treated rats; NAME = rats treated with \( \text{N}^-\text{nitro-L}-\text{arginine methyl ester (L-NAME)} \); NAME + DOCA = rats treated with DOCA plus L-NAME; NAME + OMA = rats treated with L-NAME plus omapatrilat; NAME + DOCA + OMA = rats treated with DOCA plus L-NAME plus omapatrilat; BW = body weight; LVW = left ventricular weight; KW = kidney weight; L/VW/BW = ventricular weight: body weight ratio; KW/BW = kidney weight:body weight ratio.

* \( P < 0.05 \) v control.

Values are expressed as means ± SEM.
Plasma, Renal, and Metabolic Variables

Potassium was significantly reduced in the plasma of all of the DOCA-treated groups, whereas sodium in plasma was similar in all groups. Increased plasma levels of urea and creatinine and a concomitant reduction in glomerular filtration rate (creatinine clearance) were observed in the L-NAME/DOCA group, whereas no significant differences were observed in the other groups when compared with controls (Table 2).

No significant differences were observed in food or fluid intake between any experimental group and the control group. Natriuresis and kaliuresis were not significantly different in the control and experimental groups, whereas calciuresis was significantly increased in all of the DOCA-treated groups (Table 3). Proteinuria was significantly increased in L-NAME/DOCA rats but was normalized in the L-NAME + DOCA + OMA group (Fig. 3).

Discussion

The antihypertensive effect of the simultaneous blockade of NEP and ACE was investigated in nitric oxide-deficient hypertensive rats and in these animals chronically treated with DOCA, which transforms L-NAME hypertension into an Ang II-independent model.14 The chronic administration of OMA normalized BP and prevented renal lesions in L-NAME-treated rats, suggesting that vasopeptidase inhibition has an important therapeutic effect in NO inhibition hypertension. In contrast to these results, the use of a large dose of an AT1 blocker attenuated but did not normalize BP after NO blockade.14 However, both studies are not exactly similar; the present study was of shorter duration (4 weeks) than the previous one (6 weeks).14 This may be also the reason for the absence of major renal lesions and proteinuria in the L-NAME group analyzed in the present study. In addition, it is also interesting to note that OMA exerts important preventive effects on hypertension and renal injury in L-NAME + DOCA-treated rats, which should be due to vasopeptidase inhibition, as in this experimental model, chronic Ang II blockade is completely ineffective.14

The protective effects of chronic OMA administration on BP and renal injury in the DOCA + L-NAME-treated group with low renin hypertension14 are consistent with previous observations in DOCA-salt hypertensive rats.3,5 Thus, the administration of SA7060, another dual inhibitor of NEP and ACE, efficiently prevents DOCA–salt-induced hypertension and related renal injury, mainly by inhibiting NEP, because the administration of candesartan or enalapril was less effective.5 Similar results were also observed in Dahl salt-sensitive rats treated for 8 weeks with 4% NaCl alone or in combination with either OMA (35 mg/
kg/day) or captopril (100 mg/kg/day). In these animals, the BP increase was in part prevented by concomitant OMA, while the structural changes were prevented only by OMA.

The normal values of BP in DOCA rats and the further increment in BP and renal damage produced by DOCA in NO-deficient rats agree with previous observations by our group. These observations in the L-NAME hypertensive rats treated for a longer period and with greater doses of L-NAME, 20,21 however, the L-NAME + DOCA group showed a significant increase in ventricular and renal weight relative to body weight, also in agreement with previous observations of our group. 14 The administration of OMA to L-NAME + DOCA-treated rats attenuated renal and cardiac hypertrophy, which may be secondary to the reduction on BP. However, there remains a further possibility not studied in the present work: local cardiac effects may be involved in the antihypertrophic action of OMA, as indicated by the marked reduction in absolute and relative ventricular weight in the L-NAME + OMA group in which BP was similar to controls (Table 1).

All of our groups treated with DOCA showed increased calciuria, regardless of the BP level. Chronic mineralocorticoid excess results in sodium and water retention followed by a period of escape from sodium retention, hypercalciuria, and hypertension as occurs in the DOCA-salt model. 22 Deoxycorticosterone acetate also increased BP and calciuria in the SHR model. 23 The mechanisms responsible for the increased calciuria induced by chronic mineralocorticoid excess in control and SHR are not clear. Micropuncture and clearance studies suggest that this type of hypercalciuria is produced in the terminal nephron and can be significantly reduced by hydrochlorothiazide and amiloride. 24,25 An increased renal perfusion pressure has been investigated as an important factor implicated in this alteration. 26 Hypercalciuria and hypertension are prevented in DOCA-salt treated rats when dietary NaCl is replaced by equimolar amount of NaCO3 H, 27 and hypercalciuria and hypertension are also associated in other models of clinical and experimental hypertension. 28 However, our data indicate that an increased BP per se does not produce hypercalciuria, because the DOCA group, normotensive, showed hypercalciuria and the L-NAME group, hypertensive, was normocalciuric.

In conclusion, the present results demonstrate that the
administration of OMA completely prevents the increased BP of L-NAME hypertension, and that when plasma renin activity is suppressed by DOCA in this hypertension, the increased BP and renal injury are improved by the vasodilators and natriuretic peptides induced by the simultaneous blockade of NEP and ACE. Further studies with specific blockers will be necessary to delineate the exact role that these vasodilator peptides play in this model of arterial hypertension.

Acknowledgments

We are grateful to R. Arcas and M. Quintana for expert technical assistance. We thank R. Davies for assistance with the English version. OMA was a gift from Bristol-Myers Squibb Company.

References