Prevention of the Cardiovascular and Renal Effects of Angiotensin II by Endothelin Blockade

Abderraouf Herizi, Bernard Jover, Nathalie Bouriquet, Albert Mimran

Abstract—Angiotensin II (Ang II) stimulates the release and gene expression of endothelin-1 in isolated vascular smooth muscle cells. In 47 Sprague-Dawley rats, we assessed the influence of concomitant treatment by the mixed ET<sub>A</sub>/ET<sub>B</sub> endothelin receptor antagonist bosentan (30 mg/kg per day, gavage) on the effect of a 10-day infusion of Ang II (200 ng/kg per minute, SC, osmotic pump) on arterial pressure, renal hemodynamics (microsphere method), albuminuria, cardiac weight, and carotid structure. Ang II increased systolic arterial pressure (SAP) by 49±7 mm Hg. Although bosentan alone did not affect SAP, the development of Ang II–induced hypertension was entirely prevented by the endothelin antagonist. In addition, the reduction in renal blood flow induced by Ang II (4.9±0.3 versus 7.4±0.2 mL·min<sup>−1</sup>·g<sup>−1</sup> in control rats) was prevented by concomitant administration of bosentan (8.8±0.8 mL·min<sup>−1</sup>·g<sup>−1</sup>). The marked increase in albuminuria observed in rats infused with Ang II (2524±961 versus 91±6 μg/24 h in control rats) was prevented by bosentan. Similarly, bosentan abolished the increase in heart weight index (from 2.96±0.03 to 3.41±0.08 mg/g body weight) and carotid media thickness (from 73±14 to 108±6 μm) induced by Ang II infusion. Of interest, the diuresis of Ang II was not influenced by bosentan. In conclusion, endogenous endothelin contributes to the cardiovascular and renal effects of Ang II. (Hypertension. 1998;31[part 1]:10-14.)

Key Words: endothelin ■ angiotensin II ■ hemodynamics ■ hypertrophy ■ albuminuria

In experimental animals, the chronic administration of Ang II results in a dose-dependent rise in arterial pressure, cardiac<sup>1,2</sup> and peripheral vascular hypertrophy and proliferation,<sup>3</sup> and injury of small intrarenal arterial vessels (focal fibrinoid necrosis and medial hyperplasia).<sup>4</sup> As a consequence of the increase in arterial pressure and exaggerated glomerular capillary permeability and pressure, an increase in albuminuria and the development of glomerulosclerosis are observed during chronic Ang II infusion.<sup>5</sup> Such a renal effect of Ang II mimics the elevated albuminuria often seen in renin-dependent forms of human hypertension such as that associated with renal artery stenosis and thrombosis.<sup>5</sup> Nevertheless, Ang II may have blood pressure-independent effects on target organs, as suggested by the development of cardiac hypertrophy in rats infused with a nonpressor dose of Ang II<sup>6</sup> or when the increase in arterial pressure was prevented by hydralazine.<sup>2</sup>

In recent years, it was reported that Ang II is a powerful stimulator of ET-1 release by cultured vascular smooth muscle<sup>7</sup> and endothelial cells.<sup>8</sup> In addition, Ang II stimulated the expression of preproendothelin-1 mRNA and the ET-1 gene in cultured rat<sup>9</sup> and bovine<sup>10</sup> endothelial cells, rat vascular smooth muscle cells,<sup>10</sup> cardiomyocytes,<sup>10</sup> and renal mesangial cells.<sup>11</sup> In fact, it was demonstrated that part of the hypertrophic and mitogenic effects of Ang II may be mediated by ET-1 as suggested by the influence of monoclonal antibodies to ET-1,<sup>12</sup> inhibition of endothelin-converting enzyme by phosphoramidon,<sup>7</sup> and blockade of type A endothelin receptors by BQ-123.<sup>13</sup> Vascular ET-1 may also act as an amplifier of the vasoconstrictor effect of Ang II, because in normotensive rats a dose of ET-1 devoid of pressor effect potentiated the effect of a nonpressor dose of Ang II, thus resulting in an increase in arterial pressure.<sup>13</sup>

In the present studies, the influence of the mixed ET<sub>A</sub> and ET<sub>B</sub> receptor antagonist bosentan<sup>14</sup> on the development and maintenance of hypertension as well as organ damage (kidney, heart, and large vessels) associated with chronic infusion of Ang II was assessed in rats.

Methods

Experiments were carried out in 47 male Sprague-Dawley rats (Ilıa-Credo, L’Arbresle, France) weighing 275 to 300 g and maintained on a normal sodium intake (sodium-free rat chow containing <5 mmol sodium per kg and distilled water containing 77 mmol sodium per liter as drinking fluid). Animals were placed in individual metabolic cages at least 1 week before studies and randomly assigned to the four experimental groups.

After a 3-day baseline period, Ang II or its vehicle (distilled water) was infused alone (Ang II and control groups, respectively, consisting of 13 rats each) or in association with the oral administration of bosentan (Ang II–Bos and Bos groups consisting of 13 and 8 rats, respectively). Ang II (Sigma Chemical Co) was infused subcutaneously via osmotic pumps (model 2002, Alza Corp) at a dose of 200 ng·kg<sup>−1</sup>·min<sup>−1</sup> for 10 days and bosentan (Ro 47–0203, donated by Dr Jean-Paul Clozel and Dr Martine Clozel of F Hoffmann-La Roche Ltd, Basel, Switzerland) was administered once daily (between 8 and
by guest on May 30, 2017 http://hyper.ahajournals.org/ Downloaded from

10 AM) by gavage at a dose of 30 mg/kg (in 1 mL/kg of a suspension of arabic gum), 24 hours before and during the 10-day period of Ang II infusion. In preliminary experiments, such a dose of bosentan totally blocked the vasopressor and vasodepressor responses to an intravenous bolus of ET-1 (300 pmol/kg) given 2 hours after acute bosentan and 24 hours after the last dose in rats treated by bosentan for 3 days.

Body weight, food and water intake, urine volume, and excretion of creatinine and electrolytes were measured daily, whereas urinary excretion of albumin was determined before and at the end of the experimental period. In all samples, concentrations of sodium and potassium were measured by flame photometry and creatinine concentration by a colorimetric method. Urinary excretion of albumin was determined by immuno nephelometric method.16

Results were expressed as mean±SEM and analyzed by one- or two-factor ANOVA for repeated measures as appropriate. Differences between groups were assessed by the Fisher’s protected least significant difference test. Within-group differences were evaluated by the Student’s t test for paired values. A value of P<.05 was considered statistically significant.

Results
Arterial Pressure
As depicted in Fig 1, SAP measured by the tail-cuff method in conscious rats remained stable throughout studies in the control untreated group (basal 127±1 mm Hg and final value 125±1 mm Hg) and rats treated by bosentan alone (basal 125±4 mm Hg and final value 128±3 mm Hg). In rats infused with Ang II alone, SAP rose progressively from a basal value of 124±2 mm Hg to 139±4 on day 1, 148±5 on day 3, and 173±6 mm Hg on the final day. Administration of bosentan, 24 hours before and for the duration of studies, strikingly attenuated the development of Ang II–induced hypertension. Although the final SAP level (136±2 mm Hg) was similar to baseline (130±3 mm Hg), SAP increased by 14±4 mm Hg on day 3 (P<.02), a value significantly higher than control untreated rats (P<.02) and similar to that observed in rats infused with Ang II alone (24±5 mm Hg).

Metabolic Parameters
Within the 10-day period of the study, body weight gain was significantly attenuated in the Ang II group (27±6 g) when compared with untreated (43±4 g) and bosentan-treated (43±7 g) groups. Concomitant administration of bosentan prevented the body growth impairment associated with chronic Ang II (43±5 g).

As shown in Fig 2, water intake markedly increased during Ang II administration. The dipsogenic effect of Ang II was not affected by bosentan, which otherwise had no influence on water intake when given alone in normotensive rats. At the end of studies, plasma concentration of sodium and potassium and hematocrit level were similar in all groups.

Renal Hemodynamics and Function
After recovery from ether anesthesia, intra-arterial MAP was 111±1 and 134±3 mm Hg in untreated control rats and Ang II–infused rats. Although MAP in bosentan–treated normotensive rats was similar to that in control rats (116±7 mm Hg), bosentan entirely prevented Ang II–induced hypertension (106±6 mm Hg). As depicted in Fig 3, the reduction of cardiac output and renal blood flow, as well as the increase in total peripheral and renal resistances, associated with chronic administration of Ang II, were totally prevented by concomitant administration of bosentan. Treatment of normotensive rats with bosentan had no detectable effect on systemic and renal hemodynamics.

Final serum creatinine was similar in all groups. Creatinine clearance calculated from 24-hour urine and serum creatinine values was higher in Ang II–infused rats when compared with control animals (497±27 versus 343±21 µL/min per g kidney

Selected Abbreviations and Acronyms
Ang II = angiotensin II
ET-1 = endothelin-1
MAP = mean arterial pressure
SAP = systolic arterial pressure

Analytical Methods and Statistical Analysis
In all samples, concentrations of sodium and potassium were measured by flame photometry and creatinine concentration by a colorimetric method. Urinary excretion of albumin was determined by immuno nephelometric method.15

Results were expressed as mean±SEM and analyzed by one- or two-factor ANOVA for repeated measures as appropriate. Differences between groups were assessed by the Fisher’s protected least significant difference test. Within-group differences were evaluated by the Student’s t test for paired values. A value of P<.05 was considered statistically significant.

Figure 1. Influence of bosentan on the change in tail-cuff systolic arterial pressure associated with chronic infusion of Ang II. *P<.05 vs untreated group; †P<.05 between Ang II and Ang II–bosentan groups.

Figure 2. Water intake (mL) in rats with chronic Ang II (24 days) and bosentan treatment (24 days) compared with control untreated (24 days) group.

Figure 3. Renal hemodynamics in untreated control, Ang II-infused, bosentan-treated, and Ang II–bosentan groups.
Interestingly, creatinine clearance was still higher in the group treated with Ang II and bosentan than in control animals (486±58 μL/min per g kidney wt).

As shown in Fig 4, urinary excretion of albumin markedly increased in Ang II–infused rats (from 217±47 to 2524±961 μg/24 hours, P<.01), whereas it remained constant in untreated and bosentan–treated control rats. Concomitant bosentan administration abolished the proteinuric effect of Ang II.

Cardiovascular Changes

As summarized in the Table, a significant increase in heart weight index was observed in Ang II–treated rats (3.41±0.08 versus 2.96±0.03 mg/g body wt in untreated animals, P<.05), and bosentan prevented the cardiac hypertrophic effect of Ang II. In addition, chronic treatment with Ang II was associated with a consistent incremental increase in the carotid media thickness without a change in the lumen diameter. The mean value of carotid media thickness was restored to control values in rats treated by Ang II and bosentan.

Discussion

In the present studies, the development of hypertension, as well as cardiac and vascular hypertrophy, resulting from chronic infusion of angiotensin II was prevented by the concomitant administration of the mixed endothelin receptor antagonist bosentan. Moreover, renal vasoconstriction and proteinuria associated with Ang II were similarly abolished by bosentan. Interestingly, the dipsogenic effect of Ang II was not affected by bosentan.

In previous studies conducted in renin–dependent forms of experimental hypertension, such as that observed in the early phase of partial unilateral renal ablation (ligation of two of the three branches of the left renal artery), acute administration of the endothelin receptor A antagonist BQ-123 was associated with a fall in arterial pressure similar to that induced by captopril. In rats with renal artery clipping and intact contralateral kidney, bosentan prevented approximately 40% of the early increase in arterial pressure. However, no effect of acute blockade of ET₄ receptors by FR139317 on arterial pressure was found in two-kidney, one clip hypertensive rats studied 4 weeks after clipping, and administration of bosentan 8 weeks after clipping (at a phase characterized by cardiac and vascular overexpression of the ET-1 gene) had no effect on arterial pressure or the structure of small vessels. These observations suggest a major contribution of endothelin to the increase in arterial pressure, only during the early (and probably highly

Figure 2. Influence of bosentan on the change in water intake associated with Ang II. *P<.05 vs untreated group.

Figure 3. Influence of bosentan on the effect of Ang II on systemic and renal hemodynamics measured at the end of study in conscious rats. *P<.05 vs untreated group.
Aortic rings), associated with chronic infusion of Ang II at a
rise in arterial pressure and alteration in endothelial function (as
receptor antagonist LU135252 prevented a major part of the
findings, the recent observation that the selective endothelin-A
bosentan may be of great value. In agreement with our
unpublished data); a study of the effect of a higher dose of
prevention of hypertension. Nevertheless, vasodilatation in-
cient dosage of the antagonist could explain the incomplete
stimulation of endothelin-A receptors by LU135252, insuffi-
could be attributed to blockade of the vasopressor effect of
influence of the antagonist on Ang II–induced hypertension
Figure 4. Urinary excretion of albumin measured before and at
the end of the treatment period in rats given Ang II, bosentan
alone, or a combination of the two. *P<.05 vs untreated group;
†P<.05 between Ang II–bosentan and Ang II alone.
duced by the release of nitric oxide and prostacyclin that results
from the lack of blockade of type B endothelin receptors23 may
have contributed to the effect of LU135252. Our findings
clearly suggest that endothelin markedly influenced the hyper-
tensive effect of Ang II, at least when administered at a dose of
200 ng · kg⁻¹ · min⁻¹, which was shown to result in a threefold
increase in the circulating concentration of the octapeptide.2,24
The present results, obtained in chronically treated rats, extend
previous studies showing that bosentan shifted to the right the
dose-response curve of arterial pressure and cardiac output to
acute Ang II.25 The observed protective effect of endothelin
inhibition is in favor of an important role for the stimulation of
endothelin release by Ang II, probably via activation of type 1
Ang II receptors.7,26

Whether the influence of endothelin is specific to Ang II
remains to be established. It was recently reported that chronic
norepinephrine infusion augmented the ventricular expression
of ET-1 mRNA and that bosentan prevented the development of
cardiac hypertrophy; unfortunately no measurement of
arterial pressure was reported.27 In addition, Emori et al28
observed that Ang II and vasopressin both stimulated the
release of ET-1 and the expression of preproendothelin-1
mRNA by cultured bovine endothelial cells.7

In the present studies, bosentan prevented cardiac hypertro-
phy as well as carotid artery structural changes associated with
Ang II infusion, probably as a consequence of the lack of
increase of arterial pressure. This is in agreement with previous
studies that suggested that endothelin blockade affects hyper-
tension–associated vascular remodeling only when a significant
antihypertensive effect is observed.21,29,30 Moreover, bosentan
abolished the renal vasoconstrictor effect and the increase in
albuminuria associated with chronic Ang II infusion. Among
mechanisms of the Ang II–induced increase in albuminuria are
the increase in systemic pressure, a rise in intraglomerular
capillary pressure resulting from preferential constriction of the
efferent glomerular arteriole, and an increase in the glomerular
vascular permeability to albumin and possibly other macromolecules.4
Prevention of the albuminuric effect of Ang II by bosentan
could be the consequence of the lack of increase in systemic
pressure and blockade of the renal vasoconstrictor effect of
exogenous Ang II, at least within the rather short period of
administration of the octapeptide.

<table>
<thead>
<tr>
<th>Influence of Bosentan on Cardiovascular Changes Induced by Chronic Ang II Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Initial tail-cuff pressure, mm Hg</td>
</tr>
<tr>
<td>Final tail-cuff pressure, mm Hg</td>
</tr>
<tr>
<td>Body weight gain, g/10 d</td>
</tr>
<tr>
<td>Heart weight index, mg/g body wt</td>
</tr>
<tr>
<td>Kidney weight index, mg/g body wt</td>
</tr>
<tr>
<td>Carotid cross-sectional area, mm²</td>
</tr>
<tr>
<td>Carotid media thickness, μm</td>
</tr>
<tr>
<td>Carotid lumen diameter, μm</td>
</tr>
<tr>
<td>Carotid media/lumen</td>
</tr>
</tbody>
</table>

*ND indicates not determined. Values are mean±SEM.
*P<.05 vs untreated rats, †P<.05 vs Ang II alone.
As shown in Fig 2, no influence of the endothelin antagonist on the known dipsogenic effect of Ang II$^{1–33}$ was observed. This suggests that either bosentan did not cross the blood-brain barrier and thus reach endothelin receptors located within the central nervous system or that endothelin does not contribute to the dipsogenic effect of Ang II. In fact, it was reported that intracerebroventricular injection of endothelin exerted an antidiipsogenic effect through type A receptors and that central administration of the type A–receptor antagonist BQ-123 significantly accentuated the drinking response elicited by Ang II.$^{34}$

Acknowledgments

This work was supported by the French Ministère de l’Éducation Nationale, de l’Enseignement Supérieur et de la Recherche. Bosentan was kindly provided by Dr Martine Clozel from F Hoffmann-La Roche Ltd, Basel, Switzerland.

References


Prevention of the Cardiovascular and Renal Effects of Angiotensin II by Endothelin Blockade

Abderraouf Herizi, Bernard Jover, Nathalie Bouriquet and Albert Mimran

Hypertension. 1998;31:10-14
doi: 10.1161/01.HYP.31.1.10

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/1/10

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/