Cardiovascular Effects of Combination of Perindopril, Candesartan, and Amlodipine in Hypertensive Rats

Shokei Kim, Yumei Zhan, Yasukatsu Izumi, Hiroshi Iwao

Abstract—The combination therapy with ACE inhibitors, angiotensin II type 1 (AT₁) receptor antagonists, or calcium channel antagonists may exert more beneficial effects on cardiovascular diseases than monotherapy. Perindopril, candesartan cilexetil, or amlodipine alone or the combination of low doses of each agent was administered orally to stroke-prone spontaneously hypertensive rats (SHRSP) for 4 weeks to compare the hypotensive or cardiovascular effects. Although perindopril (2 mg/kg), candesartan cilexetil (2 mg/kg), or amlodipine (3 mg/kg) alone caused comparable hypotensive effects in SHRSP, monotherapy with perindopril or candesartan decreased left ventricular (LV) weight; mRNA levels for atrial natriuretic factor, skeletal α-actin, and collagen types I and III; and aortic weight and platelet-derived growth factor-β receptor tyrosine phosphorylation to a greater extent than monotherapy with amlodipine. Although monotherapy with a low dose (0.2 mg/kg) of perindopril or candesartan cilexetil did not significantly reduce the LV mRNA levels and aortic platelet-derived growth factor-β receptor phosphorylation of the SHRSP, combination therapy at such a low dose normalized these parameters more potently than the use of amlodipine (3 mg/kg) alone. Although perindopril or candesartan cilexetil alone at 0.05 mg/kg did not decrease the blood pressure of the SHRSP, such a low dose of combination therapy decreased LV weight and atrial natriuretic factor mRNA levels of the SHRSP to a greater extent than amlodipine alone or amlodipine combined with perindopril or candesartan cilexetil. Our results provide evidence that suggests the combination of an ACE inhibitor and an AT₁ receptor antagonist may be more effective in the treatment of cardiac and vascular diseases than the combination of a calcium channel blocker with an ACE inhibitor or an AT₁ receptor antagonist or monotherapy with each agent. (Hypertension. 2000;35:769-774.)

Key Words: angiotensin ■ calcium ■ hypertrophy ■ platelet-derived growth factor ■ rats, stroke-prone SHR ■ antihypertensive therapy

Clinically, combination therapy with different types of antihypertensive drugs is very frequently used in hypertensive patients in an effort to achieve more beneficial effects than monotherapy and to diminish the incidence of unwanted side effects. Accumulating evidence indicates that ACE inhibitors and angiotensin (Ang) II type 1 (AT₁) receptor antagonists are effective in the treatment of cardiovascular diseases or congestive heart failure.1-5 Recently, in spontaneously hypertensive rats6 and the (mRen-2)27 transgenic rats,7 the combination of low doses of an ACE inhibitor and an AT₁ receptor antagonist has been shown to induce greater reductions in blood pressure and cardiac weight than monotherapy with the same or higher doses. Furthermore, the combined administration of an ACE inhibitor and an AT₁ receptor antagonist decreases blood pressure in normotensive volunteers8,9 and improves cardiac dysfunction in patients with heart failure10,11 more than either intervention alone, suggesting that combined ACE inhibitor and AT₁ receptor antagonist therapy may be more effective for the treatment of hypertension or heart failure than either agent alone. In addition to blocking the renin-angiotensin system, long-acting calcium channel blockers are reported to be effective for the treatment of cardiovascular diseases as well as hypertension.12-14 Therefore, a direct comparison between calcium channel blockers and renin-angiotensin blockers, regarding the effects on cardiovascular injuries, is clinically very important. However, there is little information on the effects on cardiovascular diseases of the combined administration of a calcium channel blocker with a renin-angiotensin blocker. Furthermore, it is unclear which combination therapy is more organ protective: a calcium channel blocker combined with a renin-angiotensin blocker or an ACE inhibitor combined with an AT₁ receptor antagonist. In the present study, we obtained evidence that suggests the combination of an ACE inhibitor and an AT₁ receptor antagonist may be more effective in the treatment of cardiovascular diseases than a calcium channel blocker combined with an ACE inhibitor or an AT₁ receptor antagonist.

Methods

Experimental Protocol

All procedures were in accordance with institutional guidelines for animal research. Stroke-prone spontaneously hypertensive rats

Received September 21, 1999; first decision October 1, 1999; revision accepted October 21, 1999.

From the Department of Pharmacology, Osaka City University Medical School, Abeno-ku, Osaka, Japan.

Correspondence to Shokei Kim, MD, PhD, Department of Pharmacology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. E-mail kims@med.osaka-cu.ac.jp

© 2000 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org

769
TABLE 1. Effects of Low Doses of Perindopril, Candesartan Cilexetil, or Amlodipine on Blood Pressure of SHRSP

<table>
<thead>
<tr>
<th>Blood Pressure, mm Hg</th>
<th>Perindopril, mg/kg</th>
<th>Candesartan Cilexetil, mg/kg</th>
<th>Amlodipine, 0.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td>Vehicle</td>
<td>0.05</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>218 ± 7</td>
<td>204 ± 6</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>215 ± 8</td>
<td>199 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>192 ± 5</td>
<td>175 ± 11</td>
</tr>
<tr>
<td>4 wk</td>
<td>Vehicle</td>
<td>221 ± 7</td>
<td>216 ± 8</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>196 ± 5</td>
<td>192 ± 5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>187 ± 7</td>
<td>174 ± 4</td>
</tr>
</tbody>
</table>

Blood pressure was measured at 1 and 4 weeks after the start of each drug treatment and at 4 to 5 hours after oral dosing of each drug or vehicle, because the maximal hypotensive effects of these drugs occurred at 4 to 6 hours after oral dosing. Values are mean ± SEM (n = 6 or 7).

*P < 0.05, †P < 0.01 vs vehicle.
effects of combination therapy on left ventricular weight and gene expression of SHRSP

As shown in Table 2, 2 mg/kg perindopril or candesartan cilexetil alone, and their combination (0.2 mg/kg each), decreased the left ventricular weight of the SHRSP to a greater extent than 3 mg/kg amlodipine alone ($P<0.01$). Notably, even the combination of 0.05 mg/kg perindopril and candesartan cilexetil, despite the less hypotensive effects, reduced left ventricular weight more potently than amlodipine alone ($P<0.01$). Furthermore, the combination of 0.5 mg/kg amlodipine with 0.2 mg/kg perindopril or candesartan cilexetil, which produced a less hypotensive effect than the use of 3 mg/kg amlodipine alone, reduced left ventricular weight as much as the use of amlodipine alone.

We examined the effects of combination therapy on cardiac hypertrophy and remodeling-associated genes, including fetal phenotype of genes (ANF and skeletal $\alpha$-actin) and collagen types I and III. These gene expressions are significantly enhanced in SHRSP compared with control normotensive WKY rats, as we previously demonstrated. In our preliminary experiments, we examined the effects of monotherapy of 0.2 mg/kg perindopril, 0.2 mg/kg candesartan cilexetil, or 0.5 mg/kg amlodipine on the cardiac mRNAs of the SHRSP and found that such low doses of each drug failed to reduce the mRNA levels (data not shown). As shown in Figure 1, high doses of monotherapy drugs or low doses of combination therapy drugs that we examined all significantly decreased left ventricular mRNA levels for ANF, skeletal $\alpha$-actin, collagen type I, and collagen type III. Interestingly, perindopril or candesartan cilexetil (2 mg/kg) or their combination (0.2 mg/kg each) decreased left ventricular ANF.

![Figure 1](image-url)
skeletal α-actin, and collagen type I mRNA levels to a greater extent than did 3 mg/kg amlodipine alone. Furthermore, although the combination of 0.05 mg/kg perindopril and candesartan cilexetil had a less hypotensive effect than 3 mg/kg amlodipine alone and had similar hypotensive effects as 0.5 mg/kg amlodipine combined with perindopril or candesartan cilexetil, this combination decreased left ventricular ANF mRNA levels to a greater extent than amlodipine alone or the combination with perindopril or candesartan cilexetil.

**Aortic PDGF and EGF Receptors in SHRSP**

As shown in Table 3, aortic PDGF-β receptor tyrosine phosphorylation in the SHRSP was significantly increased by 1.85-fold \( (P<0.01) \) compared with the control WKY rats, whereas there was no significant difference in aortic PDGF-β receptor protein levels between the SHRSP and WKY rats, indicating that aortic PDGF-β receptor activation is enhanced in SHRSP compared with WKY rats. On the other hand, there was no statistically significant difference between the SHRSP and WKY rats with respect to aortic PDGF-α receptor tyrosine phosphorylation or protein levels or EGF receptor tyrosine phosphorylation or protein levels (Table 3). Perindopril or candesartan cilexetil alone (2 mg/kg), and their combination (each 0.2 mg/kg), significantly decreased not only aortic weight (Table 2) but also aortic PDGF-β receptor tyrosine phosphorylation of the SHRSP to a comparable degree, whereas other drug treatments did not significantly affect their parameters (Figure 2). On the other hand, aortic PDGF-β receptor protein levels were not significantly affected by any drug treatments (Figure 2).

**Effect of Hoe140 on Perindopril-Treated SHRSP**

Hoe140, a bradykinin B₂ receptor antagonist, had no affect on the mentioned effects of perindopril on blood pressure; left ventricular weight; aortic weight; left ventricular mRNAs for ANF, skeletal α-actin, collagen type I, and collagen type III; and aortic PDGF-β receptor tyrosine phosphorylation in SHRSP (data not shown).

**Discussion**

Combination therapy is very frequently used in hypertensive patients. Furthermore, recent experimental or clinical evidence suggests that combination therapy of an ACE inhibitor and an AT₁ receptor antagonist is useful for the treatment of hypertension and cardiac hypertrophy or to improve cardiac function.⁶⁻¹⁰,¹¹⁻¹²,¹³ However, the effects of the combination of calcium channel blockers with an ACE inhibitor or an AT₁ receptor antagonist are poorly understood. Furthermore, it is unclear which combination therapy is more effective: the combination of an ACE inhibitor with an AT₁ receptor antagonist or the combination of a calcium channel blocker with a renin-angiotensin blocker. Therefore, in the present work, we compared the effects of combination therapy on cardiac hypertrophy and gene expression and aortic growth factor receptor activation.

Our present observations provide evidence that the combination of an ACE inhibitor with an AT₁ receptor antagonist produced hypotensive effects that were greater than those provided by the combination of a calcium channel blocker with an ACE inhibitor or an AT₁ receptor antagonist, which supports previous experimental and clinical data⁶⁻⁹ indicating that the combination of an ACE inhibitor and an AT₁ receptor antagonist may be useful for the treatment of hypertension. Cardiac hypertrophy is characterized not only by the increase in myocyte size but also by gene reprogramming such as the upregulation of fetal genes and extracellular matrix genes.²³,²⁴ Thus, it is essential for the estimation of cardiac hypertrophy to examine the effects on cardiac gene expressions. However, the effects of combination therapy on cardiac gene expression have not been examined in previous reports.⁶,⁷,²² Therefore, here, we investigated cardiac gene expressions and found that the combination of low doses of an ACE inhibitor and an AT₁ receptor antagonist (0.2 mg/kg each) decreased cardiac mRNA levels for ANF, collagen type I, and skeletal α-actin more potently than amlodipine alone. Furthermore, the combination of 0.05 mg/kg perindopril and candesartan cilexetil, whose hypotensive effects were similar to those of low doses of amlodipine combined with perindopril or candesartan cilexetil and were lesser than those of high doses (3 mg/kg) of amlodipine alone (Table 2), decreased left ventricular weight and ANF mRNA levels more potently than combination therapy or monotherapy with amlodipine. Thus, the combination of an ACE inhibitor and an AT₁ receptor antagonist may be more effective in not only regression of cardiac hypertrophy but also normalization of cardiac gene reprogramming than monotherapy or combination therapy with a calcium channel blocker.

In addition to the heart, vascular tissues are another important target for antihypertensive drugs. Previous work on the combination of an ACE inhibitor and an AT₁ receptor antagonist have not examined the effect on vascular injury.⁶⁻⁷,²² Recent in vitro reports on cultured vascular smooth muscle cells indicate that PDGF and EGF receptors play a major role in vascular smooth muscle cell growth⁵⁵ and that the growth effect of Ang II is due to transactivation of EGF

**TABLE 3. Tyrosine Phosphorylation of Aortic PDGF and EGF Receptors in 17-Week-Old SHRSP and WKY Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>PDGF-β Receptor</th>
<th>PDGF-α Receptor</th>
<th>EGF Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phospho Tyr</td>
<td>Protein Level</td>
<td>Phospho Tyr</td>
</tr>
<tr>
<td>SHRSP</td>
<td>1.85±0.11*</td>
<td>1.16±0.07</td>
<td>1.25±0.12</td>
</tr>
<tr>
<td>WKY Rats</td>
<td>1±0.04</td>
<td>1±0.05</td>
<td>1±0.07</td>
</tr>
</tbody>
</table>

Phospho Tyr indicates tyrosine phosphorylation of each receptor in aorta, estimated with immunoprecipitation followed by Western blot analysis as described in the text. Values are mean±SEM \( (n=6 \text{ or } 7) \). The mean value of WKY rats is represented as 1. *\( P<0.01 \) vs WKY rats.
receptor\(^{26}\) or PDGF receptor\(^{27}\) via AT\(_1\) receptor. However, it is still unclear whether the activation of EGF or PDGF receptors by Ang II can occur in an in vivo situation. In the present work, we obtained the first evidence that vascular PDGF-\(\beta\) receptor tyrosine phosphorylation, but not PDGF-\(\alpha\) receptor or EGF receptor tyrosine phosphorylation, was enhanced in hypertensive rats and that this enhanced PDGF-\(\beta\) receptor activation was at least in part mediated by AT\(_1\) receptor, as shown by the significant inhibitory effects of perindopril and candesartan cilexetil but not amlodipine. Interestingly, although 0.2 mg/kg perindopril or candesartan cilexetil alone failed to suppress aortic PDGF-\(\beta\) receptor tyrosine phosphorylation, their combination significantly decreased aortic PDGF-\(\beta\) receptor tyrosine phosphorylation as much as 2 mg/kg of each agent alone. Taken together with the fact that the PDGF-\(\beta\) receptor plays a central role in vascular smooth muscle cell proliferation and migration in vivo,\(^{28-30}\) these findings support the notion that combination therapy with an ACE inhibitor and an AT\(_1\) receptor antagonist may be useful for the treatment of vascular remodeling.

In the present work, either bradykinin accumulation\(^{14}\) or AT\(_2\) receptor\(^{31}\) might be responsible for the beneficial effects of the combination of low doses of perindopril and candesartan cilexetil. Our results show that treatment with Hoe140 did not affect the cardiac and aortic effects of perindopril. This observation suggests that there was no contribution of bradykinin B\(_2\) receptor to the actions of perindopril under our experimental conditions, although the possible importance of bradykinin cannot be completely ruled out.\(^{32,33}\) Furthermore, differing from an AT\(_1\) receptor antagonist alone, the combination with an ACE inhibitor is shown to suppress plasma Ang II elevation induced by AT\(_1\) receptor antagonist alone, indicating that AT\(_2\) receptor activation resulting from AT\(_1\) receptor antagonist treatment is inhibited by the combination with an ACE inhibitor. Thus, the mechanism of greater effectiveness with the combination of an ACE inhibitor and an AT\(_1\) receptor antagonist that was observed in the present work seems to be due to a more potent inhibition of Ang II–mediated AT\(_1\) receptor activation itself rather than to bradykinin accumulation or AT\(_2\) receptor activation. Thus, in vivo studies with an ACE inhibitor alone or an AT\(_1\) receptor antagonist alone may underestimate the role of the renin-angiotensin system in hypertension and in cardiovascular hypertrophy and remodeling.

In conclusion, our present observations show that the combination of low doses of an ACE inhibitor and an AT\(_1\) receptor antagonist suppresses cardiac hypertrophy–related gene reprogramming and aortic PDGF-\(\beta\) receptor activation in SHRSP.
Our findings further extend the notion that the combination of an ACE inhibitor and an AT₁ receptor antagonist may be more effective in the treatment of hypertension and cardiovascular diseases than monotherapy.6–9,21,22 Furthermore, we propose that the combination of an ACE inhibitor with an AT₁ receptor antagonist may be more effective in the treatment of cardiovascular hypertrophy and remodeling than the combination with a calcium channel blocker.

Acknowledgment
This work was supported in part by a Grant-in-Aid for Scientific Research (09470527) from the Ministry of Education, Science, and Culture.

References
Cardiovascular Effects of Combination of Perindopril, Candesartan, and Amlodipine in Hypertensive Rats
Shokei Kim, Yumei Zhan, Yasukatsu Izumi and Hiroshi Iwao

_Hypertension._ 2000;35:769-774
doi: 10.1161/01.HYP.35.3.769

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/35/3/769

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/