Angiotensin II Receptor Blockade in Normotensive Subjects
A Direct Comparison of Three AT1 Receptor Antagonists

Lucia Mazzolai, Marc Maillard, Julien Rossat, Jürg Nussberger, Hans R. Brunner, Michel Burnier

Abstract—Use of angiotensin (Ang) II AT1 receptor antagonists for treatment of hypertension is rapidly increasing, yet direct comparisons of the relative efficacy of antagonists to block the renin-angiotensin system in humans are lacking. In this study, the Ang II receptor blockade induced by the recommended starting dose of 3 antagonists was evaluated in normotensive subjects in a double-blind, placebo-controlled, randomized, 4-way crossover study. At 1-week intervals, 12 subjects received a single dose of losartan (50 mg), valsartan (80 mg), irbesartan (150 mg), or placebo. Blockade of the renin-angiotensin system was assessed before and 4, 24, and 30 hours after drug intake by 3 independent methods: inhibition of the blood pressure response to exogenous Ang II, in vitro Ang II receptor assay, and reactive changes in plasma Ang II levels. At 4 hours, losartan blocked 43% of the Ang II–induced systolic blood pressure increase; valsartan, 51%; and irbesartan, 88% (P<0.01 between drugs). The effect of each drug declined with time. At 24 hours, a residual effect was found with all 3 drugs, but at 30 hours, only irbesartan induced a marked, significant blockade versus placebo. Similar results were obtained when Ang II receptor blockade was assessed with an in vitro receptor assay and by the reactive rise in plasma Ang II levels. This study thus demonstrates that the first administration of the recommended starting dose of irbesartan induces a greater and longer lasting Ang II receptor blockade than that of valsartan and losartan in normotensive subjects. (Hypertension. 1999;33:850-855.)

Key Words: angiotensin II receptors, angiotensin II human irbesartan losartan valsartan

For the past 15 years, angiotensin-converting enzyme (ACE) inhibitors have been widely used to block the renin-angiotensin system and to treat hypertension and congestive heart failure. Despite their recognized clinical efficacy, ACE inhibitors have some weaknesses. First, ACE is a nonspecific enzyme that uses bradykinin as a substrate in addition to angiotensin I (Ang I). Thus, ACE inhibition results in both Ang I and bradykinin accumulation, the latter being a potential source of side effects such as angioedema.1,2 The occurrence of cough, the most frequent side effect of ACE inhibitors, has also been attributed to the lack of specificity of ACE inhibition.1 Second, during long-term ACE inhibition, plasma angiotensin II (Ang II) levels decrease, but some Ang II is still circulating at measurable levels.3 The reactive rise in plasma renin activity and plasma Ang I levels may account for the persistence of measurable Ang II levels, particularly if ACE activity is not fully inhibited around the clock. More recently, it has also been postulated that besides being generated through the primary ACE pathway, Ang II can be generated through non-ACE pathways that are not affected by ACE inhibitors.4

To overcome the drawbacks of ACE inhibitors, substantial effort has been put into development of new compounds that block the renin-angiotensin system in a more specific manner. In recent years, several specific, nonpeptide, orally active Ang II receptor antagonists have been developed and have become available for treatment of hypertension.5 These antagonists are as effective as ACE inhibitors, calcium channel blockers, and β-blockers in lowering blood pressure in hypertensive patients, and several large clinical trials are now under way to demonstrate their ability to lower cardiovascular morbidity and mortality.

All Ang II receptor antagonists inhibit the renin-angiotensin system by selectively blocking the AT1 subtype of Ang II receptors. When investigated in normotensive subjects, compounds such as losartan, valsartan, irbesartan, and candesartan have been shown to inhibit, in a dose-dependent manner, the blood pressure response to exogenous Ang II.6–10 Based on data obtained in healthy subjects, several doses of each compound have been tested for antihypertensive efficacy in hypertensive patients. Although the same mechanism of action applies for all Ang II antagonists, pharmacokinetic differences exist among the antagonists; these differences could potentially result in different efficacy profiles. Use of Ang II receptor antagonists is rapidly increasing; thus, information on the relative ability of different AT1 antagonists to block the renin-angiotensin system is of interest. So far, direct comparisons of AT1 receptor blockade among Ang II antagonists have been performed almost exclusively in in

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From the Division of Hypertension and Vascular Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.
Correspondence to M. Burnier, MD, Division of Hypertension and Vascular Medicine, CHUV, 1011 Lausanne, Switzerland. E-mail Michel.Burnier@chuv.hospv.ch
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vitro studies and in animal experiments and few comparisons have been conducted in vivo in humans.

The purpose of this study was to compare, in normotensive subjects, the degree and time course of the Ang II receptor blockade induced by a single recommended starting dose of losartan, irbesartan, and valsartan in a double-blind, placebo-controlled, randomized, 4-way crossover study using 3 different evaluations of Ang II receptor blockade. Our results show that a single administration of the recommended starting dose of irbesartan (150 mg) induces greater and longer lasting Ang II receptor blockade than valsartan (80 mg) and losartan (50 mg) in normotensive subjects.

**Methods**

**Subjects**
Twelve normotensive male volunteers aged 22 to 36 years (mean, 25±4 years) and weighing 66 to 89 kg (mean, 74±7 kg) were enrolled in the study. Before inclusion, all volunteers underwent a complete physical examination, a detailed medical history was taken, and routine laboratory tests were performed. After the nature and purpose of the study were explained, informed written consent was obtained from each subject. The protocol was approved by our institutional review committee.

**Study Design**
In this double-blind, randomized, placebo-controlled, 4-way crossover study, the 12 subjects were randomly selected to receive, at 1-week intervals, a single oral dose of irbesartan (150 mg), valsartan (80 mg), losartan (50 mg), or a placebo. Sodium and water intake were not restricted during the study.

Each week, subjects entered the hospital after an overnight fast and were asked to lie in the supine position. Two intravenous catheters were inserted into antecubital veins, 1 for the injection of exogenous Ang II and the other on the contralateral arm for collection of blood. After at least 30 minutes of rest, baseline blood pressure and heart rate were monitored by photoplethysmography at the finger as described previously11 and blood was taken to measure plasma Ang II and aldosterone levels. Blood was also taken to assess Ang II receptor blockade with an in vitro receptor binding assay.

**Plasma Ang II and Aldosterone Measurements**

Plasma Ang II levels were measured by an immunoreactive method using monoclonal antibody against Ang II as previously described.12

To further assess Ang II receptor blockade, a standardized, in vitro receptor binding assay was used.13 In brief, this in vitro binding assay measures the ability of a subject’s plasma to displace radiolabeled Ang II bound to AT, receptors in a rat smooth muscle cell membrane preparation. Binding is performed at 37°C for 1 hour with 100 µg of membrane proteins in 375 µL of binding buffer in the presence of 5 fmol of labeled Ang II and 25 µL of plasma. Tests with blanks are performed and the standard curve is determined by using 25 µL of a reference plasma. Nonspecific binding is estimated by adding 10 µmol/L of unlabeled human Ang II to the incubation mixture. Separation of bound, labeled Ang II is achieved by centrifugation, and residual radioactivity is determined by gamma counting. Within each assay, separate competition binding curves are determined with cold Ang II and with the AT, receptor antagonist losartan to assess the reproducibility of the method and the quality of the membranes. The amount of endogenous Ang II, which can reach 40 fmol/mL after administration of an Ang II receptor antagonist, remains very low and does not interfere with competition binding.

The coefficient of variation for repeated measurements within the same subject receiving a placebo was 5.2±0.7% (n=12). Between-assay precision ranged from 3% to 8% for plasma showing either no or some antagonistic activity.

**Statistical Analysis**

Results are mean±SE. Statistical analysis was performed using ANCOVA, P<0.05 was considered significant. The dose-response curves to exogenous Ang II were analyzed using logarithmic relationships. A log(dose)–SBP response relationship was calculated at 0, 4, 24, and 30 hours for each subject. Individual curves were characterized by their slopes and y intercepts. To establish the mean dose-blood pressure response curve at each time point, the slopes and y intercepts of each individual curve were averaged and used to reconstruct the mean curves. ANCOVA was used to analyze the drug-induced shifts of dose-response curves.

**Results**

**In Vivo Assessment of Ang II Receptor Blockade**

All drugs were well tolerated, and none of the subjects were excluded from the study. The time course of the SBP response to the top dose of exogenous Ang II in the 4 phases is presented in Figure 1. In the placebo phase, the Ang II–induced blood pressure response was constant and present at all times. Four and 24 hours after drug intake, the blood pressure response to Ang II was blocked significantly by all 3 antagonists, dose-response curves to exogenous Ang II were presented at 0, 4, 24, and 30 hours for each subject. Individual curves were characterized by their slopes and y intercepts. To establish the mean dose-blood pressure response curve at each time point, the slopes and y intercepts of each individual curve were averaged and used to reconstruct the mean curves. ANCOVA was used to analyze the drug-induced shifts of dose-response curves.

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To better characterize the receptor blockade induced by the 3 antagonists, dose-response curves to exogenous Ang II were established at each time point (Figure 2). The dose-
response curves for the placebo did not vary with time. With losartan, a significant rightward shift of the dose-response curve to Ang II was found only at 4 hours ($P < 0.05$ for the $y$ intercept but no difference in slope). With valsartan, the dose-response curve shifted significantly at both 4 hours ($P < 0.05$ for the slope and $y$ intercept) and 24 hours ($P < 0.05$ for the $y$ intercept). The most significant shifts of the dose-response relationships were observed with irbesartan. Indeed, the curves were significantly different from those obtained at baseline and from those obtained with the placebo at 4, 24, and 30 hours ($P < 0.05$ for the $y$ intercept at all time points and for the slope at 4 and 24 hours).

**In Vitro Assessment of Ang II Receptor Blockade**

The ability of the 3 Ang II receptor antagonists to block AT$_1$ receptors was also assessed in vitro using the AT$_1$ receptor radioreceptor binding assay. With this approach, the capacity of each subject’s plasma to compete with the binding of radiolabeled Ang II to AT$_1$ receptors was analyzed in a competition binding assay. In subjects receiving the placebo, no change in Ang II binding to its AT$_1$ receptor was found at 4, 24, and 30 hours (Figure 3). In contrast, in subjects receiving an AT$_1$ receptor antagonist, the binding of radiolabeled angiotensin was markedly reduced. The results of the in vitro assay are similar to those obtained in vivo. Indeed, the antagonistic activity of irbesartan was again significantly greater and longer lasting than that of either losartan or valsartan.

**Plasma Ang II Measurements**

The reactive rise in plasma Ang II levels was measured at each time point immediately before injection of exogenous radioreceptor binding assay. With this approach, the capacity of each subject’s plasma to compete with the binding of radiolabeled Ang II to AT$_1$ receptors was analyzed in a competition binding assay. In subjects receiving the placebo, no change in Ang II binding to its AT$_1$ receptor was found at 4, 24, and 30 hours (Figure 3). In contrast, in subjects receiving an AT$_1$ receptor antagonist, the binding of radiolabeled angiotensin was markedly reduced. The results of the in vitro assay are similar to those obtained in vivo. Indeed, the antagonistic activity of irbesartan was again significantly greater and longer lasting than that of either losartan or valsartan.

**Figure 1.** Inhibition of SBP to exogenous Ang II. Time course of the in vivo Ang II receptor blockade induced by 50 mg of losartan (●), 80 mg of valsartan (○), 150 mg of irbesartan (△), and placebo (□). Values are mean±SEM. *$P < 0.05$. **$P < 0.01$ vs placebo. #$P < 0.05$ vs other antagonists.

**Figure 2.** Displacement of the Ang II–blood pressure response curves. SBP response to increasing doses of exogenous Ang II before and after administration of a single dose of placebo, losartan, valsartan, and irbesartan. Dose-response curves were obtained before (square) and 4 (●), 24 (○), and 30 hours (△) after drug intake.

**Figure 3.** In vitro receptor blockade. Time course of the drug-induced Ang II receptor blockade assessed by an in vitro receptor binding assay in which the ability of plasma from subjects undergoing different treatments to displace radiolabeled Ang II was measured. Values are mean±SEM. For definitions of symbols, see legend of Figure 1.
Ang II (Figure 4). As expected, plasma Ang II levels remained stable with the administration of the placebo. Increases in circulating Ang II were observed with all 3 Ang II receptor antagonists. However, significant changes in plasma Ang II levels from baseline were found only with irbesartan and valsartan. Four hours after drug intake, the increase induced by irbesartan was significantly greater than that induced by losartan or valsartan (P<0.05).

Discussion
This study was designed to compare in humans the AT1 receptor antagonism induced by the recommended starting doses of 3 Ang II antagonists (losartan, valsartan, and irbesartan). Ang II receptor blockade was assessed with 3 independent methods. Our results show that a single dose of 150 mg of irbesartan causes a greater and more sustained blockade of AT1 receptors than a single dose of 50 mg of losartan or 80 mg of valsartan. All 3 antagonists were more effective than the placebo 4 and 24 hours after drug intake, but only irbesartan induced a significant residual blockade 30 hours after dosing.

Early pharmacodynamic studies conducted in normotensive subjects analyzed the ability of losartan, valsartan, and irbesartan to block the blood pressure response to exogenous Ang II. These studies, which were conducted to determine the minimal antagonistic dose and to define a range of doses to be used in clinical trials, demonstrated that at peak effect, 40 mg of losartan, 80 mg of valsartan, and 150 mg of irbesartan blocks the pressure effect of Ang II by 60%, 75%, and >80%, respectively. Although slightly different for losartan and valsartan, the data from this study are generally in accordance with these early findings. Indeed, the antagonistic effect of valsartan was slightly lower than that reported by Muller et al. However, in our experiment, valsartan was given after a meal, which is known to reduce gastrointestinal absorption of valsartan by >40%. This food effect may not be clinically relevant during long-term therapy in hypertensive patients but may well reduce the antagonistic activity when a single dose of valsartan is tested. The antagonism produced by 50 mg of losartan (ie, 35% to 45% blockade of AT1 receptors) was also weaker than expected on the basis of previous results of studies using 40 mg of losartan. To explain this difference, one must consider that in our study, the placebo had no effect on blood pressure response to exogenous Ang II, whereas it blunted the effect of Ang II by almost 20% in Christen et al’s study. Thus, if one corrects for the placebo effect, the percentage of inhibition obtained in the 2 studies is comparable.

At 4 hours, the entire Ang II–blood pressure dose–response curve was shifted to the right by the antagonists, indicating that the doses of Ang II could be increased several-fold without inducing any major increase in SBP. For safety reasons, however, the dose of Ang II was not always increased until a 30-mm Hg rise in SBP was achieved. Our data do not indicate whether the 3 tested antagonists caused a surmountable or insurmountable blockade of AT1 receptors in humans. However, previous in vitro studies have demonstrated that EXP3174, the active metabolite of losartan, valsartan, and irbesartan, induces an insurmountable antagonism of AT1 receptors, probably reflecting a slow dissociation of all 3 antagonists from the receptor.

When the antagonists were evaluated with an in vitro angiotensin receptor binding assay that measured the ability of a given plasma to antagonize the binding of radiolabeled Ang II in a smooth muscle cell membrane preparation, comparable results were obtained, with 150 mg of irbesartan resulting in a more pronounced and more sustained inhibition of Ang II binding. A separate, preliminary analysis of the same data demonstrated that the results obtained with this in vitro receptor binding assay correlate closely with results obtained in vivo with the administration of exogenous Ang II, particularly when only active treatment phases are considered. This indicates that the in vitro assay is a good alternative method for characterizing Ang II receptor blockade in humans. A similar assay using rat lung tissue was recently published.

Blockade of the renin-angiotensin system at the AT1 receptor level interrupts the negative feedback on renin secretion. Hence, AT1 receptor blockade leads to a reactive rise in plasma renin activity and plasma Ang II levels, which can be used as indirect markers of the degree of receptor blockade. In this study, the changes in plasma Ang II levels closely paralleled our in vivo and in vitro results. However, this assessment of receptor blockade is clearly less sensitive than the 2 other methods. The lack of sensitivity is mainly due to the fact that the responsiveness of renin secretion depends on salt intake and hence on the baseline activity of the renin-angiotensin system. In this study, sodium intake was not standardized. Nevertheless, the reactive rise in plasma Ang II levels was at all time points significantly more pronounced on administration of irbesartan.

In a previous study, Azizi et al suggested, on the basis of the reactive rise in plasma renin, that combined administration of a standard single oral dose of an ACE inhibitor and an Ang II receptor antagonist to salt-depleted normo-
tensive subjects had an additive effect on blood pressure and the reactive rise in renin. The investigators speculated that combination of an ACE inhibitor with an AT1 receptor antagonist could achieve a more complete blockade of the renin-angiotensin system than either therapeutic approach alone. In this study, 50 mg of captopril was combined with 50 mg of losartan. Our results show that all antagonists do not provide the same degree of Ang II receptor blockade, at least at the recommended starting dose, and that a greater reactive rise in plasma Ang II levels can be obtained with 150 mg of irbesartan than with 50 mg of losartan. Thus, before considering the combined used of ACE inhibitors and AT1 antagonists, one should ascertain that more complete blockade cannot be achieved with an adequate dose of an AT1 receptor antagonist alone.

The clinical relevance of the present results could be questioned in 2 ways. First, in this study, we investigated blockade of a single dose of each agent, and one could argue that during repeated administration, these agents could provide more pronounced blockade of Ang II receptors. This is only partly correct. Indeed, we previously showed that a repeated 8-day administration of various doses of losartan resulted in an increase in blockade of AT1 receptors at trough (ie, 24 hours after dosing) but not at peak effect. Thus, with our experimental conditions, repeated administration of each agent should reduce the difference at 24 hours and eventually at 30 hours but certainly not at 4 hours. Second, our study was conducted in normotensive subjects and not in hypertensive patients. Thus, one should be careful to not extrapolate the degree of Ang II receptor blockade into antihypertensive efficacy. Indeed, 50 mg of losartan, as well as 80 mg of valsartan and 150 mg of irbesartan, has been shown to reduce blood pressure effectively in mildly to moderately hypertensive patients. However, our results are in agreement with those of some clinical studies. In a comparative study, Oddoux-Stock et al21 showed that 80 mg of valsartan is as effective as 50 mg of losartan in controlling blood pressure in hypertensive patients. Moreover, Kassler-Taub et al22 and Opril et al23 recently showed that the trough blood pressure–lowering effect of irbesartan is greater than the antihypertensive effect of losartan. In these studies, however, the antihypertensive efficacy of both drugs was not compared at peak effect. However, our results and a recent observation by Belz et al24 show that the greatest difference in Ang II receptor blockade between irbesartan and losartan is at peak effect. In addition, it is conceivable that higher doses of all agents could potentially result in a more effective blockade of AT1 receptors and blunt the differences among agents. If this is indeed the case, it would suggest that the recommended starting doses of both losartan and valsartan are low compared with that of irbesartan.

In conclusion, the results of this study demonstrate, by 3 independent methods, that the degree and duration of Ang II receptor blockade induced by 150 mg of irbesartan are significantly greater than observed with 50 mg of losartan and 80 mg of valsartan in normotensive subjects. This finding may have important clinical relevance when the relative antihypertensive efficacies of these agents are compared. Moreover, because blockade of the AT1 receptor represents the mechanism of action for all Ang II receptor antagonists, the difference found here may have some important clinical implications as the role of these agents in the treatment of cardiovascular diseases becomes more completely defined over the next few years.

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