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Comparative vascular and renal tubular effects of angiotensin II receptor blockers combined with a thiazide diuretic in humans

THESE

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et présentée à la Faculté de biologie et de médecine de l'Université de Lausanne

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par

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Comparative vascular and renal tubular effects of angiotensin II receptor blockers combined with a thiazide diuretic in humans

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Madame le Professeur Stephanie Clarke
Directrice de l'Ecole doctorale

Rapport de synthèse

Comparaison des effets vasculaires et tubulaires rénaux de plusieurs antagonistes des récepteurs de l'angiotensine II en combinaison avec un diurétique thiazidique chez l'humain

Objectif : Le but de ce travail était d'investiguer si les antagonistes des récepteurs AT_1 de l'angiotensine II (ARA_2) entraînent un blocage équivalent des récepteurs au niveau vasculaire et au niveau rénal, en particulier lorsque le système rénine-angiotensine est stimulé par l'administration d'un diurétique thiazidique.

Méthode : trente volontaires masculins en bonne santé ont participé à cette étude randomisée, contrôlée, en simple insu. Nous avons mesuré les variations de pression artérielle, d'hémodynamique rénale ainsi que la réponse tubulaire rénale à une perfusion d'angiotensine II 3ng/kg/min administrée sur 1 heure. Ceci avant traitement puis après sept jours d'administration, 24 heures après la dernière dose de médicament. Nous avons comparé l'irbésartan 300 mg seul ou en association avec 12.5 ou 25 mg d'hydrochlorothiazide. (irbésartan 300/12.5 ; irbésartan 300/25). Nous avons également comparé les effets de l'irbésartan 300/25 au losartan 100 mg, au valsartan 160 mg ainsi qu'à l'olmésartan 20 mg, tous administrés avec 25 mg d'hydrochlorothiazide. Chaque participant a été randomisé pour recevoir 2 traitements de 7 jours espacés d'une période d'une semaine sans traitement.

Résultats : La réponse de la pression artérielle à l'angiotensine II exogène était bloquée >90% avec l'irbésartan 300 mg seul ou en association avec le diurétique. Il en était de même avec l'olmésartan 20/25. Par contre le blocage n'était que de 60% environ dans les groupes valsartan 160/25 et losartan 100/25. Au niveau rénal, l'angiotensine II exogène réduisait le flux plasmatique rénal de 36% en pré-traitement. Dans les groupes recevant l'irbésartan 300 mg et l'olmésartan 20 mg associés à l'hydrochlorothiazide 25 mg, la vasoconstriction rénale était bloquée presque entièrement alors qu'elle ne l'était que partiellement avec le valsartan 160/25 et le losartan 100/25 (34 et 45%, respectivement). En pré-traitement, au niveau tubulaire, l'angiotensine II exogène réduisait le volume urinaire de 84% et l'excrétion urinaire de sodium de 65 %. Les effets tubulaires n'étaient que partiellement bloqués par l'administration d'ARA_2.

Conclusion : Ces résultats démontrent que les ARA_2 aux doses maximales recommandées ne bloquent pas aussi efficacement les récepteurs AT_1 au niveau tubulaire qu'au niveau vasculaire. Cette observation pourrait constituer une justification à l'hypothèse selon laquelle des doses plus importantes d'ARA_2 seraient nécessaires afin d'obtenir une meilleure protection d'organe. De plus, nos résultats confirment qu'il y a d'importantes différences entre les ARA_2, relatives à leur capacité d'induire un blocage prolongé sur 24 heures des récepteurs AT_1 au niveau vasculaire et tubulaire.

Comparative vascular and renal tubular effects of angiotensin II receptor blockers combined with a thiazide diuretic in humans

Lionel Coltamai, Marc Maillard, Alexandra Simon, Bruno Vogt and Michel Burnier

Objective The goal of this study was to investigate whether angiotensin II receptor blockers (ARBs) induce a comparable blockade of AT_1 receptors in the vasculature and in the kidney when the renin–angiotensin system is activated by a thiazide diuretic.

Method Thirty individuals participated in this randomized, controlled, single-blind study. The blood pressure and renal hemodynamic and tubular responses to a 1-h infusion of exogenous angiotensin II (Ang II 3 ng/kg per min) were investigated before and 24 h after a 7-day administration of either irbesartan 300 mg alone or in association with 12.5 or 25 mg hydrochlorothiazide (HCTZ). Irbesartan 300/25 mg was also compared with losartan 100 mg, valsartan 160 mg, and olmesartan 20 mg all in association with 25 mg HCTZ. Each participant received two treatments with a 1-week washout period between treatments.

Results The blood pressure response to Ang II was blocked by more than 90% with irbesartan alone or in association with HCTZ and with olmesartan/HCTZ and by nearly 60% with valsartan/HCTZ and losartan/HCTZ ($P<0.05$). In the kidney, Ang II reduced renal plasma flow by 36% at baseline ($P<0.001$). Irbesartan ± HCTZ and olmesartan/HCTZ blocked the renal hemodynamic response to Ang II nearly completely, whereas valsartan/HCTZ and losartan/HCTZ only blunted this effect by 34 and 45%, respectively. At the tubular level, Ang II significantly reduced urinary volume (−84%) and urinary sodium excretion (−65%) ($P<0.01$). These tubular effects of Ang II were only partially blunted by the administration of ARBs.

Conclusion These data demonstrate that ARBs prescribed at their recommended doses do not block renal tubular AT_1 receptors as effectively as vascular receptors do. This observation may account for the need of higher doses of ARB for renal protection. Moreover, our results confirm that there are significant differences between ARBs in their capacity to induce a sustained vascular and tubular blockade of Ang II receptors. *J Hypertens* 28:520–526 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: angiotensin II, blood pressure, diuretics, human, renal, vascular

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; Ang II, angiotensin II; ARB, angiotensin receptor blocker

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Introduction

Pharmacologically, angiotensin receptor blockers (ARBs) have been characterized essentially by their ability to inhibit the blood pressure (BP) response to exogenous angiotensin I or II dose-dependently or to induce a reactive rise in plasma renin activity [1]. Clinically, however, the dose recommendations for their clinical use have been based on their antihypertensive efficacy rather than on their profile of receptor blockade. Yet, studies have demonstrated that the recommended doses of several angiotensin II (Ang II) receptor antagonists do not provide a full blockade of Ang II type 1 (AT_1) receptors around the clock and not even at peak and that there are significant differences in the time profile of Ang II receptor blockade among ARBs [2–4]. Moreover, several clinical studies have suggested that ARBs may provide clinical benefits beyond their effect on BP control [5–7]. These pressure-independent beneficial effects of ARBs have been attributed in part to the blockade of tissue renin–angiotensin systems (RAS) [7]. Thus, several investigators have proposed either to combine ARBs with an angiotensin-converting enzyme (ACE) inhibitor [8–10] or to use higher doses of ARBs than those recommended for BP control [11–14] in order to obtain a greater and longer-lasting blockade of the system and hence to provide a better organ protection.

Although the results of the Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) did not support the hypothesis of a reduced morbidity and mortality with the use of an

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association of an ARB and an ACE inhibitor [11,15,16], several recent studies have shown that the use of higher doses of ARB or a more intense blockade of the RAS induce a greater decrease in proteinuria in patients with diabetic or nondiabetic nephropathies [11–14,17,18]. The greater impact of high doses of ARBs on proteinuria may be due to the fact that Ang II is abundantly produced within the kidney and intrarenal Ang II receptors are widely distributed both on the renal vasculature and on several segments of the nephron [19].

If several studies have investigated the ability of various ARBs to block the vascular effects of Ang II in humans, no study has assessed whether the same doses of ARB are able to block the renal hemodynamic and tubular effects of Ang II as effectively. Thus, it may well be that the doses of ARB effective to inhibit the BP response to exogenous Ang II do not inhibit the intrarenal effects of the peptide as efficiently. A discrepancy between the vascular and the renal effects of ARBs may be particularly relevant when ARBs are prescribed with a thiazide diuretic in order to activate the RAS [20]. So far, the impact of combining a diuretic to ARBs on the capacity of these agents to inhibit AT_1 receptors has never been investigated in humans. Therefore, the main objectives of the present study were to investigate the impact of the coadministration of a thiazide diuretic on the vascular and renal tubular blockade of the AT_1 receptor antagonist irbesartan and to compare the vascular and the tubular blockade of AT_1 receptors induced by different ARBs combined with 25 mg of hydrochlorothiazide (HCTZ).

Participants and methods

Participants

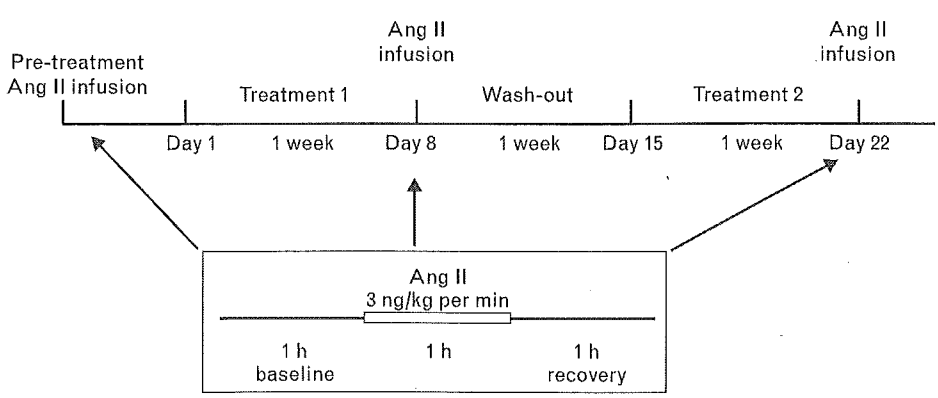
Thirty healthy, normotensive, male volunteers were enrolled in this study. Before inclusion, all volunteers underwent a complete physical examination, a detailed medical history was taken, and routine laboratory tests were performed. After explaining the nature and purpose of the study, informed written consent was obtained from each participant. The protocol was approved by the local institutional review committee (Ethical Committee of the Canton de Vaud, Lausanne, Switzerland).

Study design

In this single-blind, randomized, two-period study, participants were randomly allocated to receive two treatments for 1 week separated by a 1-week washout period as shown in Fig. 1. Following treatments were randomized: irbesartan 300 mg, irbesartan 300 mg combined with HCTZ 12.5 or 25 mg, losartan 100 mg + 25 HCTZ, valsartan 160 mg + 25 mg HCTZ and olmesartan 20 mg + 25 mg HCTZ. During the first period of treatment, the individuals were randomly assigned to irbesartan 300 mg once daily (o.d.) ($n = 10$), irbesartan 300 mg + 12.5 mg HCTZ ($n = 10$) and irbesartan 300 mg + 25 mg HCTZ ($n = 9$), enabling a comparison of the effect of adding a thiazide on the response to irbesartan. For the second period of treatment, individuals on irbesartan 300 mg were blindly switched to losartan 100 mg + 25 mg HCTZ, those on irbesartan 300 mg + 12.5 mg HCTZ to olmesartan 20 mg + 25 mg HCTZ and those on irbesartan 300 mg + 25 mg HCTZ to valsartan 160 mg + 25 mg HCTZ. Volunteers were allowed a free sodium and water intake throughout the study.

Ang II receptor blockade was assessed in all participants 1 day before the randomization and repeated at the end of each study period 24 h after the last intake of study medication to assess the drug affect at trough. On each study day, individuals entered the hospital after an overnight fast and were comfortably installed in supine position. One catheter was inserted into an antecubital vein to collect blood. A second catheter was inserted into an antecubital vein of the other arm to perfuse Ang II and para-aminohipuric acid (PAH). The vascular AT_1

Fig. 1



Study design. Ang II, angiotensin II.

receptor blockade was assessed by measuring the BP response to a 1 h infusion of exogenous Ang II (Clinalfa, Switzerland) infused at the dose of 3 ng/kg per min, a dose known to increase systemic BP by about 10–15 mmHg in healthy individuals. Blockade of renal AT_1 receptors was assessed by measuring the changes in renal plasma flow using the PAH clearance technique as well as the changes in urinary volume, sodium, potassium and endogenous lithium excretion. These parameters were measured for 1 h before the Ang II infusion, during the 1 h infusion of Ang II and for 1 h after the infusion (recovery period). The PAH clearance and the urinary electrolyte excretion were measured as reported previously [21]. Serum and urinary creatinine were measured using the Jaffe method. Endogenous lithium was measured as published previously [22]. Clearances were calculated based on the regular formula (Ux V/Px). BP, heart rate, body weight and safety assessments were performed regularly during the study days. All potential clinical and biological side effects were recorded.

Statistical analysis

Results are expressed as mean ± SD. The statistical analysis was performed using the SAS system version 8.2 (SAS Institute Inc., Cary, North Carolina, USA). Paired Student's *t*-test was performed to test the changes induced by exogenous Ang II. A *P* value of less than 0.05 was considered significant. For each individual and for each treatment phase, the response to Ang II on treatment was compared with the individual's own baseline response before any administration of the drug using a paired *t*-test. Because of the small number of individuals in each group, no direct statistical comparison within treatments was performed.

Results

Participants enrolled in this study were aged 25 ± 3 years and weighted 78 ± 8 kg (BMI 24 ± 2 kg/m^2). Their baseline BP was 119 ± 1/66 ± 1.2 mmHg and heart rate 57 ± 1.4 beats/min. Their hemoglobin was 152 ± 5 g/l, serum creatinine was 89 ± 7 μmol/l and 24-h urinary sodium excretion was 117 ± 11 mmol/24 h. All drugs were well tolerated, and no significant adverse event was recorded during the study. One individual did not complete the second period of treatment. Hence, the data of the first period were deleted as well, leading to nine individuals completing the groups, irbesartan 300 mg + 12.5 mg HCTZ and olmesartan 20 mg + 25 mg HCTZ. As data of the recovery period did not provide additional information, they will not be presented.

Vascular and tubular response to exogenous angiotensin II at baseline

The 1-h infusion of 3 ng/kg per min Ang II increased SBP and DBP by 10.9 ± 1.1 and 13.7 ± 0.75 mmHg, respectively ($n = 30$, $P < 0.001$). Renal plasma flow decreased from 685 ± 37 to 423 ± 17 ml/min ($P < 0.001$) and creatinine clearance diminished from 141 ± 7 to 130 ± 4 ml/min ($P = 0.05$). At the tubular level, Ang II significantly decreased urinary output (from 8.0 ± 0.5 to 1.3 ± 0.1 ml/min, $P < 0.001$), urinary sodium excretion (from 192 ± 15 to 64 ± 5 μmol/min, $P < 0.001$) and endogenous lithium clearance (from 22.8 ± 1.9 to 10.5 ± 0.9 ml/min, $P < 0.001$), and the fractional distal excretion of Na (FDRNa) increased from 93 ± 0.5 to 95 ± 0.5% ($P < 0.001$).

Effect of adding hydrochlorothiazide on the vascular and tubular angiotensin II, type 1 receptor blockade induced by irbesartan

The impact of irbesartan 300 mg given alone or in association with 12.5 and 25 mg of HCTZ on the BP and renal hemodynamic and tubular responses to Ang II is presented in Table 1. The BP response to Ang II was blocked completely with all three regimens with no difference between the groups. The effect of Ang II on renal plasma flow was also blocked by more than 80% with irbesartan 300 mg and irbesartan 300 mg + 12.5 mg HCTZ and irbesartan 300 mg + 25 mg HCTZ. The Ang II-induced decrease in creatinine clearance was abolished with all three regimens. Ang II significantly decreased urinary

Table 1 Effect of increasing doses of hydrochlorothiazide combined with blockade of angiotensin II, type I receptor induced by irbesartan on the vascular and renal response to angiotensin II infusion

Parameters\drug regimen	Baseline response to Ang II ($n = 30$)	Irbesartan 300 mg ($n = 10$)	Irbesartan 300 mg + 12.5 mg HCTZ ($n = 9$)	Irbesartan 300 mg + 25 mg HCTZ ($n = 10$)
Blood pressure response (mmHg)				
Systolic	10.9 ± 1.1***	0.3 ± 1.2###	−1.4 ± .18###	0.3 ± 1.1###
Diastolic	13.7 ± 0.7***	0.3 ± 0.5###	−0.3 ± 1.0###	0.4 ± 0.8###
Changes in ERPF (ml/min)	−267 ± 30***	−19.6 ± 30##	36 ± 36###	−64 ± 34##
Changes in creatinine clearance (ml/min)	−11 ± 5**	4 ± 10	15 ± 14	12 ± 8
Changes in urine output (ml/min)	−6.7 ± 0.5**	−5.2 ± 0.9**,#	−3.1 ± 1.2*	−3.8 ± 1.0**,#
Changes in urinary Na excretion (μmol/min)	−128 ± 13***	−26 ± 17##	−34 ± 17*,##	−31 ± 12*,###
Changes in lithium clearance (ml/min)	−12.3 ± 1.2***	−5.5 ± 1.4**,##	−3.1 ± 1.2*,##	−3.8 ± 1.0*,#

Ang II, angiotensin II; ERPF, effective renal plasma flow; HCTZ, hydrochlorothiazide. * $P < 0.05$ pre-Ang II vs. under Ang II on treatment. ** $P < 0.01$ pre-Ang II vs. under Ang II on treatment. *** $P < 0.001$ pre-Ang II vs. under Ang II on treatment. # $P < 0.05$ Ang II-induced change under treatment vs. change at baseline for the same individuals. ## $P < 0.01$ Ang II-induced change under treatment vs. change at baseline for the same individuals. ### $P < 0.001$ Ang II-induced change under treatment vs. change at baseline for the same individuals.

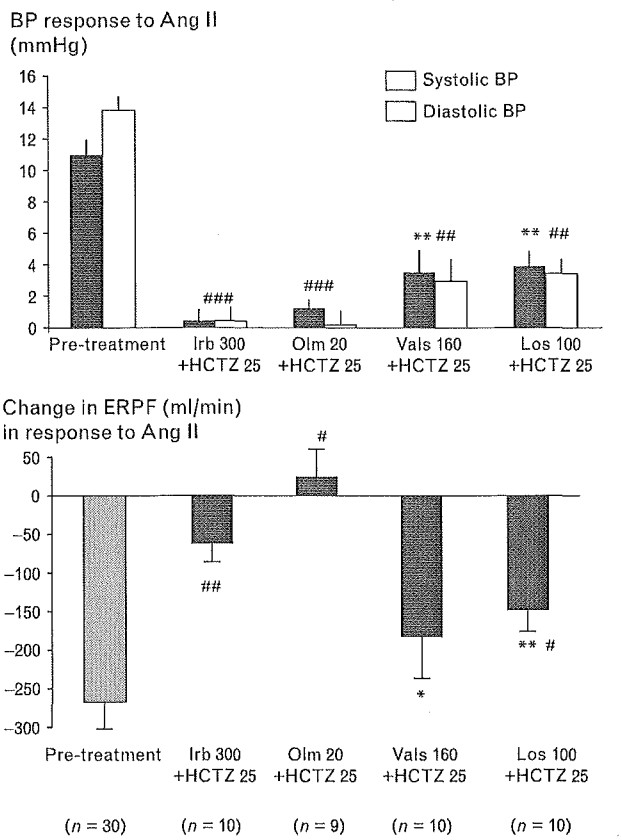
output in individuals receiving irbesartan 300 mg alone or with HCTZ whatever the dose, although the effect was blunted compared with pretreatment. Upon administration of irbesartan 300 mg, the antinatriuretic effect of exogenous Ang II was significantly reduced, but some antinatriuretic effect of Ang II was still observed in individuals receiving irbesartan and HCTZ ($P < 0.05$), although the effect was again blunted when compared with the pretreatment response. The fractional excretion of lithium (FELi) decreased significantly upon administration of Ang II. Under irbesartan alone, FELi also decreased upon infusion of Ang II ($P = 0.005$). Some significantly Ang II-induced decrease in FELi was observed in individuals receiving irbesartan combined with 12.5 and 25 mg HCTZ ($P < 0.05$ for both). Of note, after 1 week of irbesartan, FELi increased to 27%, and with the coadministration of HCTZ, FELi tended to decrease progressively with the higher dose of HCTZ (21% with 12.5 mg HCTZ and 16.6% with 25 mg HCTZ).

Comparative effects of various angiotensin II receptor blockers on the vascular and tubular response to exogenous angiotensin II

After 1 week of administration of an ARB + 25 mg HCTZ, BP did not decrease significantly with irbesartan. However, small but significant decreases in SBP were observed with losartan + HCTZ (from 119 ± 2.3 to 114 ± 2.6 mmHg, $n = 10$, $P = 0.01$ vs. pretreatment) and valsartan + HCTZ (from 119 ± 2.1 to 115 ± 2.7 mmHg, $n = 10$, $P = 0.04$). Olmesartan induced the greatest decrease in SBP and DBP from $119/69 \pm 1.5/1.5$ to $112/57 \pm 3.2/1.8$ mmHg ($n = 9$, $P = 0.05/0.01$).

The systemic and renal hemodynamic responses to Ang II in individuals receiving various ARBs in association with 25 mg HCTZ are shown in Fig. 2. At trough, the BP response to exogenous Ang II was completely blocked by irbesartan and olmesartan and to a lesser degree, although significantly, with valsartan and losartan. Almost similar results were obtained when the changes in effective renal plasma flow (ERPF) were assessed. The Ang II-induced change in ERPF was completely blocked in individuals receiving irbesartan + 25 mg HCTZ and those receiving olmesartan + 25 mg HCTZ when compared with pretreatment. In contrast, the renal hemodynamic response to exogenous Ang II was still present, though blunted, in individuals receiving losartan and valsartan + HCTZ. As shown in Fig. 3, the Ang II-induced fall in urine output was significantly blunted in individuals receiving irbesartan, olmesartan and even losartan, whereas in the valsartan group, Ang II still induced a marked decrease in urine output that was equivalent to the pretreatment response. The overall antinatriuretic effect of Ang II as well as the effect of Ang II on proximal sodium reabsorption, measured indirectly by the lithium clearance, were significantly decreased by all ARBs when combined with

Fig. 2



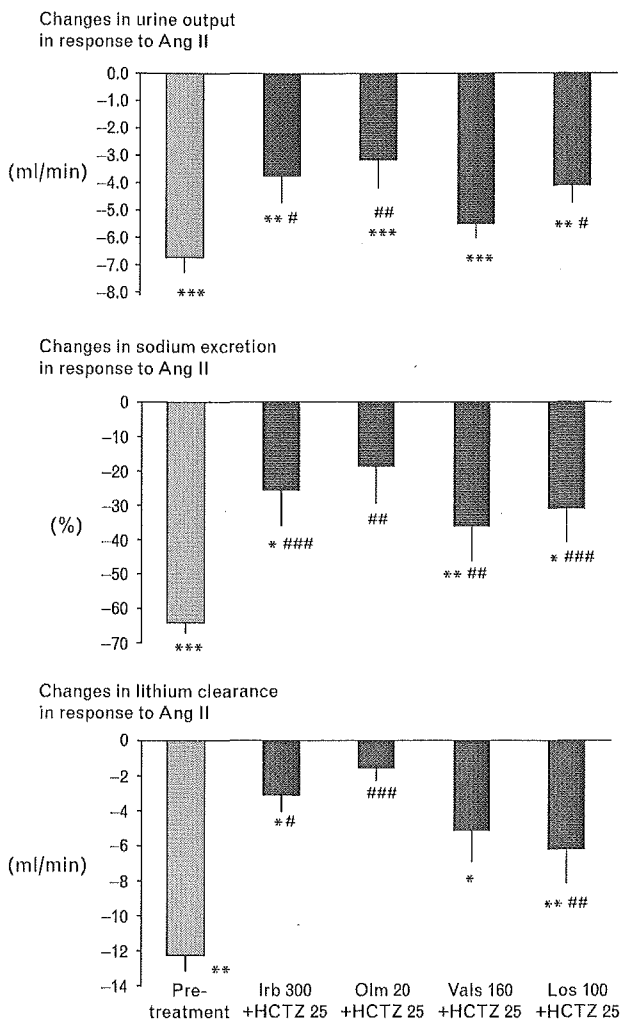
Comparative effects of irbesartan 300 mg, olmesartan 20 mg, valsartan 160 mg and losartan 100 mg, all combined with 25 mg hydrochlorothiazide on the blood pressure (upper panel) and renal hemodynamic response (ERPF) to a 1-h infusion of angiotensin II (3 ng/kg per min). $n = 30$ for the pretreatment value and $n = 9-10$ for each treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$,: pre-Ang II vs. under Ang II under treatment. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$: Ang-II-induced change under treatment vs. Ang II-induced change at baseline for the same individuals. Ang II, angiotensin II; BP, blood pressure; ERPF, effective renal plasma flow; HCTZ, hydrochlorothiazide; Irb, irbesartan; Los, losartan; Olm, olmesartan; Vals, valsartan.

25 mg HCTZ. However, the intensity of the blockade was more pronounced with irbesartan and olmesartan.

Discussion

Ang II receptor blockers have been well characterized for their ability to block the BP response to exogenous Ang II and to lower BP in hypertension [1]. Combining a thiazide diuretic with an ARB is known to increase the antihypertensive efficacy of the ARB because the diuretic induces salt depletion and BP becomes more dependent on the activity of the RAS. To our knowledge, it has never been investigated in humans whether adding a thiazide diuretic modifies the intensity of the AT_1 receptor blockade induced by ARBs. Our results show the following. First, adding a thiazide diuretic does not affect the ability of irbesartan to blunt the BP response to exogenous Ang II by more than 90%. At trough, there are, however,

Fig. 3



Comparative effects of irbesartan 300 mg, olmesartan 20 mg, valsartan 160 mg and losartan 100 mg, all combined with 25 mg hydrochlorothiazide on the angiotensin II-induced changes in urine output (upper panel), urinary sodium excretion (middle panel) and endogenous lithium clearance (lower panel). $n = 30$ for the pretreatment value and $n = 9-10$ for each treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: pre-Ang II vs. under Ang II under treatment. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$: Ang-II induced change under treatment vs. Ang II-induced change at baseline for the same individuals. Ang II, angiotensin II; HCTZ, hydrochlorothiazide; Irb, irbesartan; Los, losartan; Olm, olmesartan; Vals, valsartan.

differences between ARBs combined with HCTZ that are likely due to their pharmacological properties. Second, the blockade of the systemic BP response to Ang II and the blockade of the renal hemodynamic response to Ang II go in parallel. Our data also show that there are marked differences between ARBs in their ability to the block the vascular effects of Ang II at trough. Third, the renal tubular effects of Ang II are blunted by all ARBs independently of the presence or the absence of the thiazide. However, the intensity of blockade of the tubular effects of exogenous Ang II appears to be less complete than that obtained at the vascular level, with only 50–75% inhibition depending on the ARB. Taken together, these findings support our hypothesis that the intensity of Ang II receptor blockade induced by ARBs is not identical at the vascular and tubular level. Adding a thiazide diuretic to an ARB does not appear to have a major impact on the capacity of these agents to block AT_1 receptors. However, our results confirm previous observations, suggesting that there are differences among ARBs in their ability to block the RAS at trough.

Assessing the response to exogenous angiotensin I or II is an effective means to investigate the degree of RAS blockade induced by ACE inhibitors or Ang II receptor blockers in humans [4,23,24]. In contrast to our previous studies, Ang II was infused for 1 h at a fixed dose in order to be able to investigate its renal hemodynamic and tubular effects as well as the systemic vascular effects. As expected, Ang II induced a consistent and reproducible increase in BP, and in the kidney, Ang II significantly decreased renal plasma flow. Ang II also produced the expected decrease in urinary volume and sodium excretion, the latter being mediated essentially by an increase in proximal sodium reabsorption as reflected by the decrease in FELi.

As salt intake is known to modulate the production of renin and hence the expression of Ang II receptors [20], it appears to us of importance to examine whether a thiazide could actually modify the degree of Ang II receptor blockade induced by ARBs. In accordance with our earlier observations in the absence of diuretics [4,25], the BP response to exogenous Ang II measured 24 h after dosing was blocked by more than 90% with irbesartan and olmesartan even when combined with HCTZ, suggesting that the diuretic had little, if any, impact on the ability of ARBs to block vascular AT_1 receptors. A substantial vascular blockade was also observed with losartan and valsartan when combined with HCTZ, but the effect of these two ARBs at 24 h tended to be smaller, an observation which goes along with their known shorter half-life and their lack of 24 h AT_1 receptor blockade [1,3,4]. Of note, in this study, all drugs were prescribed at their maximal recommended doses in combination with HCTZ in our country at the time of study.

What is true for BP may not be for the renal protective effect of ARB. Thus, although the control of a high BP represents a major step to retard the progression of renal diseases, several studies have demonstrated that a more intensive blockade of the RAS results in a greater decrease in proteinuria, mainly in patients with type 2 diabetes. In this context, an intensive RAS blockade has been obtained either with the combination of an ACE inhibitor and an ARB [15,17,26] or with the use of higher doses of ARB [11–14], or more recently with the

association of an ARB and a renin inhibitor [18]. The favorable impact of higher doses of ARB on renal function might actually reflect the fact that ARB-induced AT_1 receptor blockade does not develop in parallel in the vessels and in the kidney, and that higher drug doses may be needed to block the renal RAS owing to the higher concentrations of Ang II formed in the kidney. In this respect, Vos *et al.* [27] have reported previously that the renal vasoconstrictor response to angiotensin I was maintained in volunteers receiving 20-mg enalapril twice daily, suggesting that this dose, which blocks the BP effects of angiotensin I, was not able to block the intrarenal RAS. The findings of our study further support this hypothesis. Indeed, the renal hemodynamic and tubular effects of exogenous Ang II did not appear to be blocked in parallel to the inhibition of the BP response to Ang II depending on the ARB. Thus, longer acting ARBs, such as irbesartan and olmesartan, blocked the Ang II-induced renal vasoconstriction as effectively as the BP response to Ang II, but this was clearly not the case for valsartan and losartan which induced only a partial blockade of the renal vasoconstriction, which was clearly less intense than their impact on the BP response to Ang II.

The discrepancy between the vascular and the intrarenal blockade of AT_1 receptors was even greater when considering the tubular response to exogenous Ang II. The antinatriuretic and antidiuretic effects of Ang II were blunted by all antagonists but to a lesser degree than at the vascular level. This was particularly relevant for the impact on the Ang II-induced decrease in urine output and urinary sodium excretion. This observation goes along with the measurement of greater concentrations of Ang II in the proximal segments of the nephron [19]. Of note, the addition of a thiazide diuretic was associated with a slight increase in proximal sodium reabsorption as evidenced by a lower clearance of endogenous lithium (16 ml/min) after 1 week of administration compared with pretreatment values (22.8 ml/min). This decrease may actually be the consequence of the lower systemic BP but may also be a proximal compensatory mechanism preventing an excessive loss of sodium.

Our results confirm that there are important differences between ARBs prescribed at their maximal recommended doses even when they are combined with a thiazide diuretic. The data clearly confirm our initial findings that neither valsartan nor losartan provide a 24-h blockade of AT receptors at 160 and 100 mg/day, respectively [2,3]. Since then, some studies have demonstrated that higher doses could actually improve their effect on BP and proteinuria. This is the case, for example, of valsartan, which appears to have a greater renal efficacy when used at doses up to 640 mg [12]. In our experimental setting, olmesartan 20 mg + 25 mg HCTZ had the greatest impact on BP and renal parameters, followed very closely by irbesartan 300 mg + 25 mg HCTZ.

In conclusion, ARBs do not appear to block renal AT_1 receptors as effectively as vascular receptors, and the addition of a thiazide diuretic does not affect their ability to block AT_1 receptors. Our findings are in accordance with the clinical observation that higher doses of ARB might be needed in order to lower proteinuria and to protect kidney function. Moreover, our results confirm that there are significant differences between ARBs in their ability to induce a sustained vascular and renal tubular blockade of Ang II receptors. These factors should be taken into account when designing future renal protection trials.

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