Relationship between the receptor occupancy profile and pleiotropic effects of angiotensin II receptor blockers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Angiotensin II receptor blockers (ARBs) are clinically effective in the treatment of patients with hypertension.
- ARBs exert pleiotropic effects beyond blood pressure control, including anti-oxidant and antidiabetic properties.

WHAT THIS STUDY ADDS

- The receptor occupancy profile varied among the ARBs.
- The receptor occupancy profiles of ARBs were correlated with their attenuated effect on the oxidative stress marker, but not on the markers of insulin resistance.
- ARBs might have both receptor occupancy dependent and independent pleiotropic effects.

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AIM

To investigate whether (i) angiotensin receptor occupying profiles of angiotensin II receptor blockers (ARBs) vary among the drugs and (ii) such differences contribute to the degree of their pleiotropic effects.

METHODS

In a randomized, three phase crossover study, nine hypertensive patients received repeated doses (each recommended starting dose for 7 days and then each maximum recommended dose for 20 days) of irbesartan, valsartan and candesartan. The time course profiles and trough level of receptor occupancy were determined on days 7 and 28, respectively. The pleiotropic effect related parameters were measured on days 0 and 28 in each trial.

RESULTS

Of the pleiotropic effect related parameters investigated, urinary 8-isoprostane, fasting serum insulin and homeostasis model assessment of insulin resistance index were more suppressed after 4 weeks treatment with irbesartan than after candesartan and valsartan therapy, respectively. The maximum, area under the curve and trough values of receptor occupancy significantly differed between the ARBs [geometric mean (and 95% CI) of trough value 18.1 (12.9, 25.3) for irbesartan, 9.6 (6.0, 15.3) for valsartan and 5.5 (2.8, 10.8) for candesartan, respectively] and were negatively correlated with the change in urinary 8-isoprostane ($r = -0.46 - -0.55$, $P < 0.05$), but not the markers of insulin resistance ($r = 0.02-0.15$, $P = 0.46-0.94$).

CONCLUSIONS

Our results demonstrate that the receptor occupying profiles are different among the ARBs. This class of drugs might have both receptor occupancy dependent and independent pleiotropic effects.

Introduction

Angiotensin II receptor blockers (ARBs) are recommended as first line agents for the treatment of hypertensive patients, especially with heart failure, renal dysfunction or diabetes [1]. A number of studies have indicated their pleiotropic effects beyond blood pressure control, including their anti-oxidant [2–6], anti-inflammatory [4, 6–9] and antidiabetic properties [8, 10, 11]. These effects are thought to be exerted via the blockade of angiotensin II type 1 receptors in various cell types, such as smooth muscle cells, monocytes and adipocytes, because angiotensin II mediates oxidative stress and inflammation at least *in vitro* and in animal models [12].

Several ARBs are now available for clinical use in many countries, and it has been suggested that the degree of pleiotropic effects of ARBs in the clinical setting may be different among the drugs. For example, a subset of ARBs (irbesartan, valsartan, telmisartan and olmesartan) markedly reduce serum concentrations of C-reactive protein (CRP), a marker for systemic inflammation, whereas the suppressive effect of other ARBs (candesartan and losartan) is inconsistent in spite of their obvious effects on blood pressure [13]. Taken together, these findings indicate that the profiles of angiotensin II receptor blockade differ among the drugs, and that such differences influence the pleiotropic effects more strongly than blood pressure lowering effects. However, it remains uncertain whether the profiles of angiotensin II receptor blockade differ among ARBs, because usual pharmacokinetic and pharmacodynamic studies cannot directly assess these points. Therefore, the primary aim of this study was to compare the profiles of angiotensin II receptor occupancy after repeated dosing of three ARBs, irbesartan, valsartan and candesartan, in hypertensive patients in a randomized, crossover trial. The angiotensin II receptor occupancy was determined using an *ex vivo-in vitro* radioligand receptor assay for measuring total active concentrations of ARBs including active metabolites in plasma. The secondary aim was to evaluate the relationships between the occupancy profiles of ARBs and changes in the pleiotropic effect related parameters.

Methods

Patients

A total of 10 Japanese male patients with mild hypertension [14] were recruited from the outpatient clinic at Moka Hospital (Moka, Japan) based on the following inclusion criteria: (i) aged between 20 to 64 years, (ii) either untreated patients whose clinic blood pressures were $\geq 140/90$ mmHg but $<160/100$ mmHg or patients whose clinic blood pressures were inadequately controlled ($\geq 130/85$ mmHg) but $<160/100$ mmHg in spite of treatment with a calcium channel blocker for at least 3 months and (iii) all

comorbid conditions being stable. Patients with diabetes, chronic kidney disease, three or more risk factors, target organ damage or cardiovascular disease, who are categorized as high risk [14], were excluded. We also excluded patients with moderate to severe liver disease or anaemia and those treated with any antihypertensive agents other than a calcium channel blocker or any drugs which may profoundly affect drug metabolism.

All patients provided written informed consent before participating and completed the study. Data from nine patients (aged 41–58 years, body mass index $23.5\text{--}29.2\text{ kg m}^{-2}$) were analyzable, because one patient violated the protocol. Two patients were smokers and four patients received concurrent therapy with a drug (efonidipine 40 mg day^{-1} , $n = 3$, rosuvastatin 2.5 mg day^{-1} , $n = 1$) before and during the study period.

Study design

This study was approved by the ethics committee of Jichi Medical University (Shimotsuke, Japan) and was conducted in accordance with the Declaration of Helsinki. The number in the University Hospital Medical Information Network clinical trials registry (Tokyo, Japan) was UMIN000003502.

This study was performed in an open-label, randomized, three period crossover design with a washout interval of at least 4 weeks. The patients took 100 mg irbesartan (one 100 mg Irbetan tablet, Shionogi & Co., Osaka, Japan), 80 mg valsartan (one 80 mg Diovan tablet, Novartis Pharma, Tokyo, Japan) or 8 mg candesartan (one 8 mg Blopress tablet, Takeda Pharmaceutical Co., Osaka, Japan), which are the recommended starting doses in Japan, once daily in the morning for 7 days (days 1–7). Thereafter, they took 200 mg irbesartan (two 100mg Irbetan tablets), 160 mg valsartan (one 160 mg Diovan tablet) or 12 mg candesartan (one 12 mg Blopress tablet), which are the maximum recommended doses in Japan, for 20 days (days 8–27). On days 0 and 28, venous blood and urine samples for the measurements of the pleiotropic effect parameters were collected, and sitting blood pressure at rest was measured using a mercury sphygmomanometer, in the morning after an overnight fast. On day 7, the patients fasted overnight, took the drug(s) (an ARB alone in six patients and an ARB + 40 mg efonidipine in three patients) with ~ 500 ml water, and had a standardized meal at 4 h after dosing. Venous blood samples to determine the receptor occupancy were taken immediately before and at 2, 4, 10 and 24 h after the dosing of each ARB. The plasma, serum and urine samples were stored at -80°C until analysis.

Radioligand receptor assay

To assess the profiles of angiotensin II receptor occupancy of the ARBs, the previously reported, *in vitro* radioligand receptor assay [15] was used with some modifications. Briefly, plasma (samples obtained and controls), buffer

(50 mM Tris-HCl, 5 mM MgCl₂, 1 mM EDTA, 0.05% BSA, pH 7.4), radioligand (angiotensin II (Sar¹, Ile⁸), [¹²⁵I]Tyr⁴; PerkinElmer, MA, USA), and membrane suspension (human angiotensin AT₁ receptor, PerkinElmer, MA, USA) were incubated for 90 min at 25°C. The free radioligand was then separated from the receptor bound portion by filtration through polyethyleneimine-treated Whatman GF/C filters. The filters were washed with ice-cold buffer, dried and counted for 60 s in a γ-counter.

The relative decrease of the radioligand bound to the receptors was expressed as a percentage of 100%-bound radioligand. The binding data were mathematically converted to concentration equivalents and the receptor occupancy was calculated by use of the equation:

$$\text{Receptor occupancy (\%)} = (C_f / K_i) / [(C_f / K_i) + 1] \times 100$$

where C_f is the concentration of free form of the substance and K_i is inhibition constant of the substance.

Measurement of the pleiotropic effect related parameters

Serum concentrations of insulin, high molecular weight adiponectin and interleukin-6 were measured by chemiluminescent enzyme immunoassays using reagents purchased from Fujirebio (Tokyo, Japan). Circulating concentrations of total plasminogen activator inhibitor-1 and high sensitivity CRP were assayed using latex-enhanced nephelometry kits (LPIA-tPAI test, Mitsubishi Chemical Medience, Tokyo and N-latex CRP II, Siemens Healthcare Diagnostic, Tokyo, respectively). Serum tumour necrosis factor-α and monocyte chemoattractant protein-1 concentrations and urinary 8-isoprostane and 8-hydroxydeoxyguanosine concentrations were determined using Quantikine HS human TNF-α/TNFSF1A and Quantikine human CCL2/MCP-1 immunoassay kits (R&D Systems, MN), an 8-isoprostane EIA kit (Cayman Chemical, MI) and a New 8-OHdG Check ELISA kit (Japan Institute for the Control of Aging, Nikken SEIL, Fukuroi, Japan), respectively. The other variables were measured by standard methods. The inter- and inter-assay coefficients of variation were all ≤15%. The homeostasis model assessment of insulin resistance (HOMA-IR) [16] was calculated by the following formula:

$$\text{HOMA-IR} = \text{insulin } (\mu\text{U mL}^{-1}) \times \text{glucose } (\text{mg dL}^{-1}) / 405$$

Data analysis

Noncompartmental methods were used for analysis of the receptor occupancy profiles. The maximum receptor occupancy ($RO_{\max}$), time to maximum receptor occupancy ($t_{\max}$) and trough levels of receptor occupancy (RO_{trough}) were determined directly from the observed data. The area

under the receptor occupancy–time curve from 0 to 24 h (AURO(0,24 h)) was calculated by the trapezoidal rule.

A crossover analysis of variance was used for the determination of differences in the parameters among the drugs. The statistical model included terms for sequence, treatment and period. The log-transformed data were used for the analysis of the receptor occupancy profiles. The paired *t*-test was used for pairwise comparisons when the crossover analysis of variance showed a significant drug effect. Values of $P < 0.05$ were considered to be statistically significant. All calculations were performed using WinNonlin version 5.2.1 (Pharsight, Mountain View, CA).

Results

As shown in Table 1, blood pressure and the pleiotropic effect related parameters on day 0 did not significantly differ among the three ARB periods. In addition, the study period or drug dosing order did not significantly affect any parameters of the receptor occupancy profile and the pleiotropic effects (data not shown).

The receptor occupancy profiles assessed after the seventh dosing of each ARB (the recommended starting dose in Japan, once daily in the morning) were different among the three drugs (Figure 1 and Table 2). $RO_{\max}$ was significantly higher and terminal half-life of receptor occupancy ($t_{1/2,z}$) was significantly longer after dosing with irbesartan (100 mg day⁻¹) than after dosing with valsartan (80 mg day⁻¹) (Table 2). Candesartan (8 mg day⁻¹) showed the distinct profiles with the lowest values of $RO_{\max}$ and AURO(0,24 h) and the longest values of $t_{\max}$ and $t_{1/2,z}$ (Table 2). Interestingly, although the maximum recommended dose of each ARB was used during the following period (day 8 to day 27), RO_{trough} of valsartan and candesartan were not elevated compared with the respective levels on day 8. On the other hand, RO_{trough} of irbesartan tended to increase from 12.5% on day 8 to 18.1% on day 28. Consequently, RO_{trough} on day 28 after dosing with irbesartan was significantly higher than that after dosing with other ARBs (Table 2).

As for the pleiotropic effect related parameters, the percent change in urinary concentration of 8-isoprostane, a marker of oxidative stress [17], during the 28 day period was significantly correlated with RO_{trough} on day 28 (Table 3 and Figure 2A). Additionally, the change in this marker was also correlated with the $RO_{\max}$ and AURO(0,24 h) on day 7 (Table 3). In accord with this finding, urinary 8-isoprostane concentration was more suppressed by irbesartan than by candesartan (Table 4). Other parameters investigated in this study were not significantly correlated with either RO_{trough} on day 28, or $RO_{\max}$ and AURO(0,24 h) on day 7 (Table 3). However, the following insulin sensitivity related parameters, fasting serum insulin concentration and HOMA-IR, were more suppressed after irbesartan than after valsartan (Table 4 and Figures 2B and 2C), suggesting

Table 1

Blood pressure and the pleiotropic effect related parameters before treatment with irbesartan, valsartan and candesartan (on day 0)

	Irbesartan	Valsartan	Candesartan	P value (ANOVA)
Blood pressure				
Systolic blood pressure (mmHg)	138 ± 20	137 ± 20	136 ± 23	0.98
Diastolic blood pressure (mmHg)	95 ± 17	92 ± 17	92 ± 17	0.93
Markers of oxidative stress				
Urinary 8-isoprostane (pg mg ⁻¹ creatinine)	153 ± 64	141 ± 46	140 ± 49	0.86
Urinary 8-OHdG (ng mg ⁻¹ creatinine)	10.6 ± 3.4	9.7 ± 2.3	12.4 ± 6.4	0.41
Markers of glucose tolerance				
Plasma glucose (mmol l ⁻¹)	5.55 ± 0.72	5.61 ± 0.89	5.50 ± 0.67	0.95
Serum insulin (pmol l ⁻¹)	49.0 ± 17.1	39.0 ± 12.8	46.2 ± 21.7	0.48
HOMA-IR	1.73 ± 0.66	1.42 ± 0.55	1.64 ± 0.90	0.65
Adipocytokines and inflammatory markers				
Serum adiponectin (µg ml ⁻¹)	3.5 ± 1.2	3.4 ± 1.2	3.7 ± 1.3	0.90
Plasma PAI-1 (ng ml ⁻¹)	37 ± 18	59 ± 71	36 ± 19	0.46
Serum TNF-α (pg ml ⁻¹)	1.1 ± 0.4	1.2 ± 0.4	1.1 ± 0.5	0.88
Serum MCP-1 (pg ml ⁻¹)	275 ± 110	268 ± 92	275 ± 77	0.98
Serum IL-6 (pg ml ⁻¹)	1.0 ± 0.5	1.7 ± 1.7	1.3 ± 0.8	0.42
Serum hs-CRP (ng ml ⁻¹)	407 ± 287	568 ± 550	496 ± 536	0.77

Data are mean ± SD. 8-OHdG, 8-hydroxydeoxyguanosine.

HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumour necrosis factor-α.

Figure 1

Time course of the receptor occupancy in patients with hypertension after the seventh dose of irbesartan (●, 100 mg day⁻¹), valsartan (○, 80 mg day⁻¹), and candesartan (▲, 8 mg day⁻¹). Mean ± SD, *n* = 9. **P* < 0.01 vs. candesartan (repeated measures analysis of variance with Bonferroni post hoc test)

that irbesartan reduced insulin resistance through a mechanism other than angiotensin II receptor blockade.

Discussion

A previous study has shown the receptor occupancy profiles assessed after sixth dosing of irbesartan (150 mg

day⁻¹), valsartan (80 mg day⁻¹) and losartan (50 mg day⁻¹) in healthy subjects [15]. In this study, we have shown for the first time the receptor occupancy profiles of ARBs in patients with hypertension under clinical conditions. Our data after 7 days of therapy with valsartan (80 mg day⁻¹) were similar to those in healthy subjects [15]. As expected, the mean receptor occupancy at 2 h was higher after 150 mg irbesartan in Belz's study (90.0%) [15] than after 100 mg irbesartan in our study (75.3%), suggesting that the *RO*_{max} of irbesartan dose-dependently increases. In addition, the *RO*_{trough} of irbesartan was also dose-dependent in this study. On the other hand, the *RO*_{trough} levels of valsartan and candesartan did not elevate although their doses increased. More remarkably, the receptor occupancy profile of candesartan was quite different from those of irbesartan and valsartan. Thus, these results suggest that the receptor occupancy profiles vary among ARBs.

Various clinical studies have revealed the pleiotropic effects of ARBs. For example, repeated treatments with valsartan and candesartan were reported to decrease urinary 8-isoprostane concentration, a reliable index of lipid peroxidation [17, 18], in patients with hypertension [2–6]. Because angiotensin II stimulates NAD(P)H oxidase activity, increases expression of NAD(P)H oxidase subunits, decreases several oxygen radical scavengers and consequently increases lipid peroxidation, ARBs are thought to decrease urinary 8-isoprostane concentration through inhibiting these actions of angiotensin II [18]. Decreases in the inflammatory markers were also reported during ARB therapy [4, 6–9]. However, such beneficial effects were not detected in this study. This might reside in the short dura-

Table 2

Parameters of the receptor occupancy after the repeated dosing of irbesartan (100 mg for 7 days and 200 mg for 20 days), valsartan (80 mg for 7 days and 160 mg for 20 days), and candesartan (8 mg for 7 days and 12 mg for 20 days)

	Irbesartan	Valsartan	Candesartan	P value (ANOVA)	Significantly different pair
Day 7					
RO_{max} (%)	75.3 (69.6, 81.5)	65.6 (62.1, 69.3)	21.5 (16.4, 28.1)	<0.001	All pairs
t_{max} (h)	2 (2–2)	2 (2–4)	4 (2–10)	<0.001	Irbesartan vs. candesartan, valsartan vs. candesartan
$t_{1/2,z}$ (h)	9.9 (7.7, 12.8)	7.3 (6.1, 8.6)	16.3 (13.8, 19.2)	<0.001	All pairs
AURO(0,24 h) (%·h)	765 (666, 880)	797 (729, 870)	327 (229, 467)	<0.001	Irbesartan vs. candesartan, valsartan vs. candesartan
Day 8					
RO_{trough} (%)	12.5 (8.8, 17.8)	8.5 (6.1, 11.9)	9.0 (6.2, 13.0)	0.19	
Day 28					
RO_{trough} (%)	18.1 (12.9, 25.3)	9.6 (6.0, 15.3)	5.5 (2.8, 10.8)	<0.01	Irbesartan vs. valsartan, irbesartan vs. candesartan

Data are geometric mean (95% CI), except for t_{max} , which is given as median (range). AURO(0,24h), area under the receptor occupancy–time curve from 0 to 24 h; RO_{max} , maximum receptor occupancy; RO_{trough} , trough level of receptor occupancy; $t_{1/2,z}$, terminal half-life of receptor occupancy; t_{max} , time to maximum receptor occupancy.

Table 3

Correlations between the parameters of receptor occupancy and percent changes in the pleiotropic effect related parameters after treatment with the ARBs

	RO_{trough} on day 28	RO_{max} on day 7	AURO(0,24 h) on day 7
Markers of oxidative stress			
Urinary 8-isoprostane	−0.46 (0.02)	−0.55 (< 0.01)	−0.54 (< 0.01)
Urinary 8-OHdG	−0.09 (0.65)	−0.26 (0.19)	−0.25 (0.21)
Markers of glucose tolerance			
Plasma glucose	0.18 (0.36)	−0.10 (0.59)	−0.11 (0.59)
Serum insulin	0.02 (0.92)	0.03 (0.89)	0.15 (0.46)
HOMA-IR	0.04 (0.84)	0.02 (0.94)	0.13 (0.51)
Adipocytokines and inflammatory markers			
Serum adiponectin	−0.21 (0.30)	−0.05 (0.82)	−0.09 (0.65)
Plasma PAI-1	−0.23 (0.25)	−0.20 (0.32)	−0.16 (0.42)
Serum TNF- α	0.09 (0.65)	−0.33 (0.10)	−0.33 (0.10)
Serum MCP-1	0.10 (0.61)	−0.17 (0.41)	−0.22 (0.27)
Serum IL-6	−0.12 (0.54)	−0.10 (0.61)	−0.23 (0.25)
Serum hs-CRP	−0.21 (0.30)	−0.30 (0.13)	−0.35 (0.07)

Data are correlation coefficients (P value). 8-OHdG, 8-hydroxydeoxyguanosine; ARB, angiotensin II receptor blocker; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF- α , tumor necrosis factor- α .

tion of the study, because the changes in these parameters were usually evaluated after 2 months or more of therapy in previous studies. Moreover, differences in the characteristics of participants may be another reason. Previous studies [3–6] examined hypertensive patients who had comorbid diseases, such as diabetes and metabolic syndrome. Nomura *et al.* [19] have shown that 8 weeks of treatment with valsartan alleviated hypoadiponectinaemia in hypertensive patients with diabetes, but did not elevate plasma adiponectin to supernormal levels in non-diabetic patients. Because we excluded patients with a high risk of cardiovascular events from this study, the parameters investigated were almost normal in most of the participants even before the ARB therapy (Table 1). Nevertheless, the results obtained in this study suggest that acute influences on a subset of the parameters can vary among ARBs.

Specifically, urinary 8-isoprostane and the markers of insulin sensitivity (serum insulin, HOMA-IR) increased after the 4 weeks of treatment with candesartan and valsartan, respectively. Although the underlying mechanisms for these elevations are quite unclear, various factors, including the above mentioned differences in the study duration and patient characteristics and the crossover design with washout periods, might be involved. Nevertheless, such increases were not detected after treatment with irbesartan.

Although candesartan exhibited the lower RO_{max} and AURO(0,24 h) than irbesartan and valsartan, blood pressure lowering effects were equivalent among these ARBs (Table 4). Candesartan is reported to be an inverse agonist of the angiotensin II type 1 receptor, which is defined as an agent stabilizing the receptor in an inactive conformation

Figure 2

Relationships between the trough levels of receptor occupancy on day 28 and % changes of urinary 8-isoprostane (A), serum insulin (B) and HOMA-IR (C) during the 4 week treatment period with irbesartan (●), valsartan (○), and candesartan (▲)

Table 4

% changes in blood pressure and the pleiotropic effect related parameters after 4 weeks of treatment with the ARBs

	Irbesartan	Valsartan	Candesartan	P value (ANOVA)	Significantly different pair
Blood pressure					
Systolic blood pressure	−9.7 (−14.3, −5.1)	−6.9 (−14.8, 1.1)	−9.9 (−16.5, −3.3)	0.70	
Diastolic blood pressure	−9.2 (−14.6, −3.9)	−5.6 (−12.4, 1.3)	−6.8 (−10.7, −2.9)	0.55	
Markers of oxidative stress					
Urinary 8-isoprostane	−31.7 (−78.3, 15.0)	−3.2 (−49.8, 43.4)	56.5 (9.9, 103.1)	<0.05	Irbesartanvs.candesartan
Urinary 8-OHdG	−13.2 (−86.9, 60.4)	30.4 (−43.3, 104.0)	54.3 (−19.4, 127.9)	0.39	
Markers of glucose tolerance					
Plasma glucose	1.6 (−2.6, 5.8)	1.9 (−2.3, 6.1)	2.7 (−1.6, 6.9)	0.93	
Serum insulin	−6.0 (−29.0, 17.1)	39.9 (16.9, 63.0)	9.3 (−13.8, 32.4)	<0.05	Irbesartanvs. valsartan
HOMA-IR	−4.2 (−26.2, 17.8)	40.1 (18.1, 62.1)	11.7 (−10.3, 33.7)	<0.05	Irbesartanvs. valsartan
Adipocytokines and inflammatory markers					
Serum adiponectin	−6.3 (−19.4, 6.9)	−4.3 (−17.4, 8.8)	1.4 (−11.7, 14.6)	0.66	
Plasma PAI-1	22.0 (−35.9, 79.9)	19.3 (−38.6, 77.1)	56.0 (−1.9, 113.9)	0.58	
Serum TNF-α	−15.3 (−38.0, 7.4)	−19.8 (−42.5, 2.9)	16.2 (−6.5, 38.9)	0.06	
Serum MCP-1	−1.5 (−14.9, 12.0)	0.8 (−12.6, 14.3)	4.9 (−8.5, 18.4)	0.77	
Serum IL-6	23.8 (−26.1, 73.6)	−20.0 (−69.9, 29.9)	25.9 (−24.0, 75.8)	0.32	
Serum hs-CRP	20.3 (−123.6, 164.2)	−25.1 (−169.0, 118.8)	116.1 (−27.8, 260.0)	0.34	

Data are mean (95% CI). 8-OHdG, 8-hydroxydeoxyguanosine; ARB, angiotensin II receptor blocker; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor-α.

[20], and this action might contribute to the blood pressure lowering effect of the drug observed in this study. On the other hand, the receptor inhibitory effects of irbesartan and valsartan were markedly diminished within 5 h after washout *in vitro* [21]. Unfortunately, the analysis of the receptor occupancy used in this study cannot provide any information about either the inverse agonism or dissociation profile. However, our data indicate that at least one of the pleiotropic effects of ARB, reflected by the suppression of urinary 8-isoprostane, depends on the receptor occupying action of ARB *in vivo*.

Irbesartan is a more potent agonist for peroxisome proliferator-activated receptor (PPAR) γ than valsartan and

candesartan *in vitro* [22]. Irbesartan can induce PPAR γ activity even in angiotensin II type 1 receptor-deficient cells and promotes 3T3-L1 adipocyte differentiation [23]. Although the potential clinical benefits of the PPAR γ agonistic action of irbesartan remains to be determined, the difference in the degree of this action might contribute to the different effects of ARBs on the insulin sensitivity markers observed in this study.

In summary, this study showed that the receptor occupancy profiles of irbesartan, valsartan and candesartan were different in patients with hypertension. The influences on the markers of oxidative stress and insulin sensitivity also varied among the ARBs, although the blood

pressure lowering effect did not differ. Interestingly, there was a significant negative correlation between the effect on the oxidative stress marker and the receptor occupancy profile. Further studies are needed to examine whether the receptor occupancy profile affects true endpoints of ARB therapy.

Competing Interests

There are no competing interests to declare.

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