Full Paper

Irbesartan Prevents Metabolic Syndrome in Rats via Activation of Peroxisome Proliferator-Activated Receptor γ Shinji Takai^{1,*}, Denan Jin¹, and Mizuo Miyazaki¹¹Department of Pharmacology, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki City, Osaka 569-8686, Japan

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Abstract. Irbesartan, an angiotensin-receptor blocker, is a known agonist of peroxisome proliferator-activated receptor (PPAR) γ . In this study, thirteen-week-old spontaneously hypertensive (SHR)/NDmcr-cp rats, representing a genetic model of metabolic syndrome, were treated daily with placebo, irbesartan (30 mg/kg), valsartan (10 mg/kg), or pioglitazone (10 mg/kg) for 4 weeks. Significant reductions in systolic blood pressure were seen in the irbesartan- and valsartan-treated groups, but not in the pioglitazone-treated group. Compared with the placebo group, plasma insulin, homeostasis model assessment of insulin resistance index, and plasma triglyceride levels were significantly lower while plasma adiponectin levels were significantly higher in the pioglitazone- and irbesartan-treated groups, but not in the valsartan-treated group. Significant increases in the gene expression of adiponectin and GLUT4 within adipose tissue were also observed in the pioglitazone- and irbesartan-treated groups, but not in the valsartan-treated group. These findings suggest that through PPAR γ stimulation along with angiotensin II inhibition, irbesartan may be an optimal treatment option in the prevention of metabolic syndrome as well as hypertension.

Keywords: angiotensin II, blocker, metabolic syndrome, peroxisome proliferator-activated receptor (PPAR) γ

Introduction

Metabolic syndrome represents a constellation of metabolic abnormalities including abdominal obesity, hypertension, hyperlipidemia, and diabetes. Angiotensin II is a crucial peptide for regulating blood pressure via rapid and slow pressor responses. The rapid response consists of direct contraction of peripheral resistance arteries, while the slow response consists of augmented renal sodium reabsorption via stimulation of aldosterone release from the adrenal cortex. Additionally, angiotensin II directly induces organ damage by increasing oxidative stress and levels of several growth factors. Therefore, angiotensin II-blocking agents such as angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme (ACE) inhibitors have been shown to be useful in lowering blood pressure as well as preventing cardiovascular and renal dysfunction (1).

By increasing insulin resistance, angiotensin II may

also be involved in the development of diabetes. In fact, a number of studies have shown that angiotensin II-blocking agents prevent the development of type 2 diabetes mellitus in patients with hypertension (2, 3). In high-risk Japanese hypertensive patients, candesartan-based regimens were associated with a 36% relative risk reduction of diabetes incidence in comparison to amlodipine-based regimens (4). The diabetes risk reduction with candesartan was even more prominent among the subgroup of subjects with a BMI ≥ 25.0 kg/m², where a 48% reduction in diabetes incidence was noted (4). Thus, blockade of angiotensin II may be particularly useful in preventing the development of diabetes in overweight or obese patients. Therefore, recent guidelines for the management of hypertension, such as those derived by the Japanese Society of Hypertension 2009 (5), suggest ARBs and ACE inhibitors as the first-line therapy in hypertensive patients with the metabolic syndrome.

A peroxisome proliferator-activated receptor (PPAR) γ is a nuclear receptor of ligand-activated transcription and is a crucial regulator of adipogenesis and insulin resistance in adipose tissue. The PPAR γ -activator, thiazolidinedione, lowers blood glucose level by increasing

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insulin sensitivity. Several reports demonstrated that some types of ARBs have a partial agonistic activity on PPAR γ (6, 7). For example, irbesartan enhances adipogenesis and stimulates adiponectin formation through PPAR γ activation in mouse 3T3-L1 and human adipocytes (6, 8). Further, in line with the known effects of pioglitazone, irbesartan has also been shown to lower plasma triglyceride level in patients and animal models (9–12). On the other hand, telmisartan, another PPAR γ -agonist ARB, was shown to be superior to losartan and valsartan in the improvement of insulin resistance and serum triglyceride levels in obese and hypertensive rats (13, 14). However, in these studies, the reduction of blood pressure for these ARBs was not controlled. All ARBs are well known to improve insulin resistance, but it has been unclear whether ARBs with PPAR γ -agonist properties, such as irbesartan and telmisartan, are useful for reducing insulin resistance *in vivo*.

In the present study, we sought to clarify whether despite similar hypotensive effects a PPAR γ -activating ARB is more effective at improving insulin resistance and plasma triglyceride levels than a non-PPAR γ -activating ARB. Specifically, we evaluated the effects of two ARBs: irbesartan (PPAR γ -activating) and valsartan (non-PPAR γ -activating), along with pioglitazone in the early stages of a genetic model with metabolic syndrome [spontaneously hypertensive (SHR)/NDmcr-cp rats]. Using SHR/NDmcr-cp rats, irbesartan and valsartan were compared at doses that showed the same hypotensive effects in a preliminary study.

Materials and Methods

Animals

Thirteen-week-old male SHR/NDmcr-cp rats were obtained from Japan SLC, Inc. (Shizuoka). Forty-eight SHR/NDmcr-cp rats were orally administered placebo ($n = 8$), irbesartan (30 mg/kg per day, $n = 6$), valsartan (10 mg/kg per day, $n = 6$), or pioglitazone (10 mg/kg per day, $n = 6$) by gavage, once a day for 4 weeks. In the placebo-treated rats, the same volume of solvent (0.1% carboxymethylcellulose in water) was administered. Systolic blood pressure (SBP) was monitored by tail-cuff plethysmography (BP-98; Softron Co., Tokyo) (15). After 4 weeks, the body weights of the rats were measured. The animals were anesthetized with 35 mg/kg of sodium pentobarbital administered intraperitoneally after the rats had been deprived of food for 12 h. Then, blood samples were obtained for measuring insulin, glucose, and triglyceride levels, and the adipose tissues from inguinal white fat and the liver were obtained for measuring the mRNA levels of adiponectin and GLUT4 in the adipose tissues and that of PPAR α in the liver. The experi-

mental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

Blood and plasma parameters

Blood glucose level was determined using a standard glucometer (Antosense II; Daikin Industries, Osaka). Plasma insulin level was determined by a Shibayagi Rat Insulin ELISA kit (Shibayagi, Gunma). The Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR) index was used as an indicator of insulin sensitivity according to the following formula: [fasting insulin (μ U/mL) $\times$ fasting glucose (mg/dL) / 405]. Plasma adiponectin levels were measured by a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo). Plasma triglyceride level was determined using a Wako L-Type TG H test (Wako Diagnostics, Osaka).

Real-time polymerase chain reaction (RT-PCR)

The total RNA (1 μ g) of adipose tissue or liver was transcribed into cDNA with Superscript III reverse transcriptase and random hexamers (Invitrogen, Carlsbad, CA, USA) (15). The mRNA was measured by RT-PCR on a LightCycler with specialized software (Roche Diagnostics, Tokyo) using TaqMan fluorogenic probes. All primers and probes for RT-PCR of adiponectin, GLUT4, PPAR α , and GAPDH were designed by Roche Diagnostics. The primers were 5'-tggtcacaatgggataccg-3' (forward) and 5'-cccttaggaccaagaacacct-3' (reverse) for adiponectin, 5'-gaagccaggagcttgatgtg-3' (forward) and 5'-tcagggcccctaaagtcag-3' (reverse) for GLUT4, 5'-tgcg gactaccagtcttaggg-3' (forward) and 5'-gctggagaggg tgtctgt-3' (reverse) for PPAR α , and 5'-aatgtatccgttggtga tctga-3' (forward) and 5'-gcttcaccaccttcttgatgt-3' (reverse) for GAPDH. The probes were 5'-cctggaga-3' for adiponectin, 5'-gactccag-3' for GLUT4, 5'-ccaggctc-3' for PPAR α , and 5'-cctggaga-3' (forward) for GAPDH. The mRNA levels of adiponectin, GLUT4, and PPAR α were normalized to that of GAPDH.

Statistical analyses

Data are expressed as the mean $\pm$ standard error of the mean (S.E.M.). Statistical analyses were performed using a parametric test with Fisher's Protected Least Significant Difference (StatView software for Windows; SAS Institute, Cary, NC, USA). *P*-values of < 0.05 were considered statistically significant.

Results

Body weight and blood pressure

Prior to treatment, the average body weights of rats in the placebo-, pioglitazone-, irbesartan-, and valsartan-

treated groups were 387 ± 7.2 , 387 ± 6.1 , 387 ± 7.1 , and 387 ± 6.3 g, respectively. Although no differences in body weight were observed between the placebo-, irbesartan-, and valsartan-treated groups, body weight was significantly higher in the pioglitazone- than in the placebo-treated group at 2 and 4 weeks after treatment initiation (Fig. 1A).

At baseline, SBP in the placebo-, pioglitazone-, irbesartan-, and valsartan-treated groups was 159 ± 2.8 , 161 ± 2.2 , 160 ± 3.1 , and 161 ± 3.1 mmHg, respectively. SBP in the irbesartan- and the valsartan-treated groups was significantly lower than that in the placebo group, and there was no significant difference between these two groups at 2 and 4 weeks after the treatment. On the other hand, the SBP was not different between the placebo- and pioglitazone-treated groups throughout the experiment (Fig. 1B).

Liver weight and ratio of liver weight to body weight

Liver weight was significantly lighter in the pioglitazone- and irbesartan-treated groups than in the placebo-treated group, but there was no significant difference between the placebo- and valsartan-treated groups (Fig. 2A).

Ratio of liver weight to body weight was also significantly lower in the pioglitazone- and irbesartan-treated groups than in the placebo-treated group, but that in the valsartan-treated group was not (Fig. 2B).

Blood glucose, plasma insulin and HOMA-IR index

Post-treatment, plasma glucose levels were comparable among all the treatment groups (Fig. 3A). On the other hand, plasma insulin was significantly lower in the pioglitazone- and irbesartan-treated groups than in the placebo-treated group (Fig. 3B). However, plasma insulin between the placebo- and valsartan-treated groups was not significantly different (Fig. 3B).

HOMA-IR index was significantly lower in the piogli-

tazone- and irbesartan-treated groups than in the placebo-treated group, while no significant difference was observed between the placebo- and valsartan-treated groups (Fig. 3C).

Adiponectin and triglyceride in plasma

Plasma adiponectin was significantly higher in the pioglitazone- and irbesartan-treated groups than in the placebo-treated group (Fig. 4A). On the other hand, plasma adiponectin was similar between the valsartan- and the placebo-treated groups (Fig. 4A).

Plasma triglyceride level was significantly lower in the pioglitazone-treated group (Fig. 4B). Although the effect of irbesartan on lowering plasma triglyceride level was more modest than that of pioglitazone, a significant difference in plasma triglyceride levels between the placebo- and irbesartan-treated groups was observed (Fig. 4B). Finally, the triglyceride levels were similar between the placebo- and valsartan-treated groups (Fig. 4B).

mRNA levels of adiponectin and GLUT4 in adipose tissue

Adiponectin and GLUT4 mRNA levels within adipose

Fig. 2. Liver weight (A) and ratio of liver weight to body weight (B) in the placebo (Pla)-, pioglitazone (Pio)-, irbesartan (Irb)-, and valsartan (Val)-treated groups at 4 weeks. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo-treated group.

Fig. 1. Body weight (A) and SBP (B) in the placebo (open circles)-, pioglitazone (closed circles)-, irbesartan (closed triangles)-, and valsartan (closed squares)-treated groups at 0, 2, and 4 weeks. ** $P < 0.01$ vs. the placebo-treated group.

Fig. 3. Blood glucose (A), plasma insulin (B), and HOMA-IR index (C) in the placebo (Pla)-, pioglitazone (Pio)-, irbesartan (Irb)-, and valsartan (Val)-treated groups at 4 weeks. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo-treated group.

Fig. 4. Plasma adiponectin (A) and plasma triglyceride levels (B) in the placebo (Pla)-, pioglitazone (Pio)-, irbesartan (Irb)-, and valsartan (Val)-treated groups at 4 weeks. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo-treated group.

Fig. 5. Adipose gene expressions of adiponectin (A) and GLUT4 (B) in the placebo (Pla)-, pioglitazone (Pio)-, irbesartan (Irb)-, and valsartan (Val)-treated groups at 4 weeks. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo-treated group.

tissue were significantly higher in the pioglitazone- and irbesartan-treated groups in comparison to all others (Fig. 5). However, there was no significant difference between the placebo- and valsartan-treated groups in either adiponectin or GLUT4 mRNA levels (Fig. 5).

mRNA level of PPAR α in liver

The hepatic PPAR α mRNA level was significantly higher in the pioglitazone- and irbesartan-treated groups than in the placebo-treated group, but there was no significant difference between the placebo- and valsartan-treated groups (Fig. 6).

Discussion

SHR/NDmcr-cp rats are an increasingly established animal model of metabolic syndrome that have been shown to spontaneously develop obesity, hypertension, hyperlipidemia, hyperglycemia, and hyperinsulinemia (16–18). At 5 weeks of age, the body weight and blood pressure of SHR/NDmcr-cp rats were similar to those of

Fig. 6. Hepatic gene expression of PPAR α in the placebo (Pla)-, pioglitazone (Pio)-, irbesartan (Irb)-, and valsartan (Val)-treated groups at 4 weeks. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo-treated group.

the Wistar-Kyoto rats (WKY). Thus, this stage is thought to represent a time prior to the development of the metabolic syndrome (16). On the other hand, by 17–18 weeks, body weight, blood pressure, plasma triglyceride, blood glucose, and plasma insulin were all significantly higher in SHR/NDmcr-cp than in WKY at 17–18 weeks of age. Thus, this stage is thought to represent a time after the development of metabolic syndrome (17, 18). In the present study, we used SHR/NDmcr-cp rats at an early stage of metabolic syndrome development (13 weeks). We evaluated the effects of each treatment for a 4-week duration, during which the metabolic syndrome should become developed.

The body weight of the placebo group was slightly elevated throughout the experiment, while SBP remained stable at approximately 160 mmHg. Rats treated with pioglitazone (a full PPAR γ agonist) were significantly heavier than those in the placebo-treated group. Although thiazolidinediones are known to increase body weight, the increase of body weight was not observed during treatment with irbesartan (a partial PPAR γ agonist) (19, 20). The increase in body weight after treatment with thiazolidinediones is thought to be dependent on inducing food intake and food efficacy. Although we did not measure food intake and food efficacy, increased food intake, food efficacy, or both might be observed in the pioglitazone-treated group.

This difference may be explained by the agonistic strength of the two agents, as pioglitazone can have an effect on PPAR γ at a lower concentration than irbesartan (7). On the other hand, both irbesartan and valsartan, but not pioglitazone, produced a significant hypotensive effect. However, irbesartan, like pioglitazone, significantly lowered HOMA-IR index and plasma triglycerides while valsartan did not. Although the PPAR γ -agonistic effect of irbesartan might be weaker than that of pioglitazone, our findings suggest that irbesartan may prevent insulin resistance and hypertriglyceridemia via activation of

PPAR γ rather than blockade of angiotensin II.

PPAR γ is predominantly expressed in adipose tissue and also at low levels in skeletal muscle and liver. Thiazolidinediones are known to elicit adiponectin gene expression in adipose tissues and increases plasma adiponectin levels (21–23). In the present study, we observed significant pioglitazone-induced increases of plasma adiponectin and its gene expression in adipose tissue. Several studies have shown that pioglitazone increases plasma adiponectin levels by increasing the translation of adiponectin rather than its gene expression in adipose tissue (24, 25). Although the cause of the up-regulation of plasma adiponectin cannot be determined in this study, the general changes are in agreement with previous studies (21–23).

In our study, irbesartan increased the gene expression of adiponectin as well as plasma adiponectin levels, and in contrast, valsartan had no impact. Adiponectin is an adipose-specific plasma protein and possesses insulin sensitizing properties (24). Overexpression of adiponectin in fat tissue increases plasma adiponectin and improves insulin sensitivity in mice (25). Plasma adiponectin is lower in diabetic patients than in non-diabetic patients (24, 26). Furthermore, plasma adiponectin is correlated with insulin sensitivity in non-diabetic individuals (27). Thus, plasma adiponectin is a predictor of insulin sensitivity and the development of type 2 diabetes (28). Adiponectin increased the ability of insulin to maximally stimulate glucose uptake through GLUT4 gene expression and increased GLUT4 recruitment to the plasma membrane in mouse 3T3-L1 (29). In the present study, irbesartan improved the HOMA-IR index and upregulated plasma adiponectin and adipose GLUT4 gene expression, changes that were not observed in the valsartan-treated group. The effect of irbesartan on insulin resistance might be driven by PPAR γ activation and associated augmentation of insulin-stimulated glucose uptake by GLUT4.

In the present study, pioglitazone as well as irbesartan significantly lowered plasma triglyceride level, whereas valsartan did not. In addition to improving insulin sensitivity, thiazolidinediones have been shown to reduce plasma triglyceride level in diabetic rodent models (30, 31). Plasma triglyceride level is regulated by the gut and liver-derived triglyceride-rich protein secretion and lipoprotein lipase (LPL)-mediated clearance. The promoter of LPL houses a functional peroxisome proliferator response element, through which thiazolidinediones increase the hydrolysis of triglyceride-rich lipoproteins via upregulation of LPL gene expression and fatty acid uptake in adipose tissue (32–34). Therefore, the mechanism by which irbesartan reduces plasma triglyceride level may depend on PPAR γ activation. In the present

study, PPAR α was also upregulated by irbesartan and pioglitazone, as reported in previous papers (35, 36). PPAR α is predominantly expressed in the liver, where it has a crucial role in regulating fatty acid oxidation, and its activators, such as fibrates, are known to reduce plasma triglyceride levels (37). Thus, PPAR α activation by irbesartan may be involved in the amelioration of plasma triglyceride levels in the present study. Other factors may also contribute to the reduction of plasma triglyceride level. In obese Zucker rats, irbesartan also reduced plasma triglyceride level along with upregulation of adiponectin gene expression in adipose tissue (38). Administration of adiponectin has been shown to improve glucose tolerance as well as plasma triglyceride levels (39, 40). In genetically obese diabetic mice, adiponectin administration increased gene expression of enzymes involved in β -oxidation, and these alterations decreased tissue triglyceride levels, associated with decreased plasma triglyceride level. Thus, the reduction of plasma triglyceride level in the current study in response to irbesartan treatment may be mediated by the increase in adiponectin. Nevertheless, a recent study in obese hypertensive rats reported a reduction of plasma triglyceride level by irbesartan without a concomitant increase of plasma adiponectin (35). Further studies are needed to clarify the mechanism of reduction of plasma triglyceride level by irbesartan.

On the other hand, valsartan had no effect on plasma triglyceride level in the present study. Our findings are in agreement with prior studies, which have found that losartan and olmesartan, neither of which have any impact on PPAR γ , did not reduce serum triglyceride level in obese Zucker rats (41, 42). Furthermore, serum triglyceride level did not decrease in angiotensin II type 1 receptor-deficient mice treated with a high-fat diet (43). These findings suggest that the mechanism by which irbesartan reduces plasma triglyceride level may be independent of the blockade of angiotensin II function.

In the present study, we clearly demonstrated that a PPAR γ -agonistic ARB was more useful for reduction of insulin resistance and plasma triglycerides than a non-agonistic ARB, even at doses producing an equivalent hypotensive effect. Although we did not determine the tissue triglyceride level, increased tissue triglyceride level has been reported to interfere with insulin-stimulated activation of phosphatidylinositol 3-kinase and subsequent translocation of GLUT4 and uptake of glucose, which leads to insulin resistance (44). Therefore, the reduction of plasma triglyceride level by irbesartan may also contribute to the prevention of insulin resistance.

In this study, irbesartan and valsartan were used as PPAR γ -activating and non-PPAR γ -activating ARBs, respectively. Irbesartan was derived from losartan, and

both ARBs have similar structures with biphenyl-tetrazole and imidasole groups (45). However, Fujino et al. (45) demonstrated that irbesartan has a cyclopentyl group instead of the chloride group found in losartan, and this difference in the molecular structures of these ARBs may be responsible for their different effects, determining whether or not they are PPAR γ -activating or non-PPAR γ -activating ARBs. On the other hand, valsartan has a biphenyl-tetrazole group, but no imidasole groups. Goebel et al. (46, 47) also demonstrated the structural characterization of a PPAR γ -activating ARB, telmisartan, and they showed that valsartan has no structure that docks to PPAR γ . In general, all ARBs have similar structures, but a small structural difference may be important with respect to the PPAR γ -activating property.

We also observed that the ratio of liver weight to body weight was significantly lower in the irbesartan- and pioglitazone-treated groups than in the placebo-treated group. The model used in the present study was early stage, and typical histological changes of fatty liver, such as lipid droplets, were not observed on analysis of liver slices (data not shown). Although histological changes were not observed in liver sections, the reduction of the ratio may indicate the preventive effects of these agents with respect to fatty liver. To compare the effects of irbesartan and valsartan against fatty liver, the chronic stage of the metabolic syndrome model should be used, and further study is needed.

In conclusion, irbesartan prevented the development of insulin resistance and hypertriglyceridemia and increased the gene expression of adiponectin and GLUT4 within adipose tissue of SHR/NDmcr-cp rats. Thus, irbesartan may be useful in preventing the development of metabolic syndrome due to its dual properties as a PPAR γ agonist and an angiotensin II antagonist.

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