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Irbesartan increased PPARγ activity in vivo in white adipose tissue of atherosclerotic mice and improved adipose tissue dysfunction

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ABSTRACT

The effect of the PPARγ agonistic action of an AT_1 receptor blocker, irbesartan, on adipose tissue dysfunction was explored using atherosclerotic model mice. Adult male apolipoprotein E-deficient (ApoEKO) mice at 9 weeks of age were treated with a high-cholesterol diet (HCD) with or without irbesartan at a dose of 50 mg/kg/day for 4 weeks. The weight of epididymal and retroperitoneal adipose tissue was decreased by irbesartan without changing food intake or body weight. Treatment with irbesartan increased the expression of PPARγ in white adipose tissue and the DNA-binding activity of PPARγ in nuclear extract prepared from adipose tissue. The expression of adiponectin, leptin and insulin receptor was also increased by irbesartan. These results suggest that irbesartan induced activation of PPARγ and improved adipose tissue dysfunction including insulin resistance.

1. Introduction

It has been reported that overload of carbohydrate feeding or obesity induces enlargement of adipocytes, a reduction of adiponectin secretion and thereby, an insulin-resistant condition [1–3]. Such adipocyte dysfunction might be closely related to atherosclerotic changes observed in metabolic syndrome. It is also reported that the activation of PPARγ or a PPARγ agonist, such as thiazolidines, induces adipocyte differentiation and a smaller size of adipocytes, and improves insulin resistance [1–4]. Recent reports indicated that some AT_1 receptor blockers (ARBs) show an agonistic action on a nuclear receptor, PPARγ [5–7]. We have reported that adipose tissue in an atherosclerotic model, apolipoprotein E-deficient (ApoEKO) mouse, showed an increase in adipose tissue weight, adipocyte size and expression of adipocyte differentiation factors [8]. Such changes in adipose tissue of ApoEKO mice appeared to be reciprocally regulated by AT_1 and AT_2 receptor-mediated signals [8,9]. These results suggest the possibility that ARBs showing partial agonistic activity of PPARγ could improve adipocyte dysfunction through activation of PPARγ. However, the effect of ARBs on PPARγ activity in adipose tissue has not yet been well established. In the present study, we examined the effect of irbesartan, an ARB with PPARγ agonistic activity, on DNA-binding activity of PPARγ in adipose tissue in vivo and on adipose tissue dysfunction using atherosclerotic ApoEKO mice.

2. Materials and methods

2.1. Animals and treatment

We used male apolipoprotein E (ApoE) knockout mice (ApoEKO; B6.129P2-*Apoe*^{tm1Unc}, The Jackson Laboratory, Bar Harbor, ME; based on C57BL6/J strain). The mice were treated with irbesartan (50 mg/kg/day: provided by Shionogi & Co. Ltd., Osaka, Japan) mixed in a high-cholesterol diet (HCD: 1.25% cholesterol and 10% coconut oil in MF, prepared by Oriental Yeast Co. Ltd., Tokyo, Japan) for 4 weeks from 9 weeks of age. These animals were housed in plastic cages at 25 ± 1 °C with lighting on from 7:00 to 19:00. All experimental procedures were approved by the Animal Studies Committee of Ehime University.

2.2. DNA-binding activity of PPARγ in adipose tissue

Nuclear extract from adipose tissue was prepared using a nuclear extract kit (Sigma–Aldrich, Japan) after homogenization of adipose tissue with a glass homogenizer according to the manufacturer's protocol. DNA-binding of PPARγ was determined with a DNA-binding ELISA kit (TransAM™ PPARγ, Active Motiv, Carlsbad, CA). The kit is designed to detect PPARγ protein in nuclear extract which binds to PPARγ response element immobilized at the bottom of the 96-well plate, using specific antibodies conjugated to horseradish peroxidase. Five micrograms of protein from

Abbreviations: ApoEKO, apolipoprotein E deficient; AT_1, angiotensin II type 1; ARB, AT_1 receptor blocker; PPAR, peroxisome proliferator-activated receptor; HCD, high-cholesterol diet; GLUT, glucose transporter.

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each nuclear extract was applied to the assay. Nuclear extract was also prepared from liver, and DNA-binding of PPARγ was determined for comparison.

2.3. Quantitative reverse-transcription polymerase chain reaction (RT-PCR)

Tissue samples from ApoEKO and AT_1a/ApoEKO mice were taken for measurement of adiponectin, PPARγ, leptin, insulin receptor, insulin receptor substrate 1 (IRS-1) and glucose transporter type 4 (GLUT4). RNA was extracted from epididymal and retroperitoneal adipose tissue through the use of TRI reagent and a filter cartridge according to the manufacturer's protocol (RiboPure™ Kit, Ambion, Life Technologies Japan, Tokyo, Japan). Total RNA in the aorta was extracted with an RNA extraction kit (Sepasol-RNA I Super, Nakalai Tesque, Inc., Kyoto, Japan). Quantitative real-time RT-PCR was performed using Premix Ex Taq™ (Takara Bio Inc., Shiga, Japan) as previously described [8]. The level of target gene expression was normalized against the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in each sample.

2.4. Statistical analysis

All values are expressed as means ± SEM. The effects of the different treatments on all data were evaluated with analysis of variance (ANOVA). When a significant effect was found, the results were further compared with Bonferroni's multiple range tests. A difference with $p < 0.05$ was considered significant.

3. Results

3.1. Effect of irbesartan on white adipose tissue weight and adipocyte number in atherosclerotic ApoEKO mice treated with high-cholesterol diet (HCD)

Administration of irbesartan at 50 mg/kg/day for 4 weeks did not affect feeding and body weight of atherosclerotic ApoEKO mice treated with HCD (feeding: 3.4 ± 0.2 and 3.3 ± 0.3 g/day, and body weight: 29.5 ± 0.7 and 28.8 ± 0.3 g for control and irbesartan group, respectively). In these mice, adipose tissue weight was decreased by irbesartan both in epididymal and retroperitoneal adipose tissue (Fig. 1A). On histological examination, adipocyte size appeared smaller in the irbesartan group (Fig. 1B). As shown in the bar graph, adipocyte number per unit area was increased after treatment with irbesartan (Fig. 1B). Systolic blood pressure was not significantly altered by this dose of irbesartan (data not shown).

3.2. Expression and DNA-binding activity of PPARγ in white adipose tissue of ApoEKO mice treated with HCD

Fig. 3 shows mRNA expression (Fig. 2A) and DNA-binding activity (Fig. 2B) in white adipose tissue of ApoEKO mice. Treatment with irbesartan increased the expression of PPARγ in white adipose tissue (Fig. 2A). A previous report indicated that irbesartan could increase transcription activity of PPARγ in vitro [5]. We examined DNA-binding activity of PPARγ in vivo. In wild type (C57BL/6J) mice, DNA-binding activity of PPARγ was ca. 4-fold higher in epididymal white adipose tissue than in liver (0.157 ± 0.011 and 0.033 ± 0.004 arbitrary units for adipose tissue and liver, respectively). Based on this result, we next measured DNA-binding activity of PPARγ in white adipose tissue of ApoEKO mice (Fig. 2B). Compared with the value in wild type mice, DNA-binding activity of PPARγ in white adipose tissue appeared to be decreased in ApoEKO mice. Treatment with irbesartan increased DNA-binding activity of PPARγ in both epididymal and retroperitoneal adipose tissue (Fig. 2B).

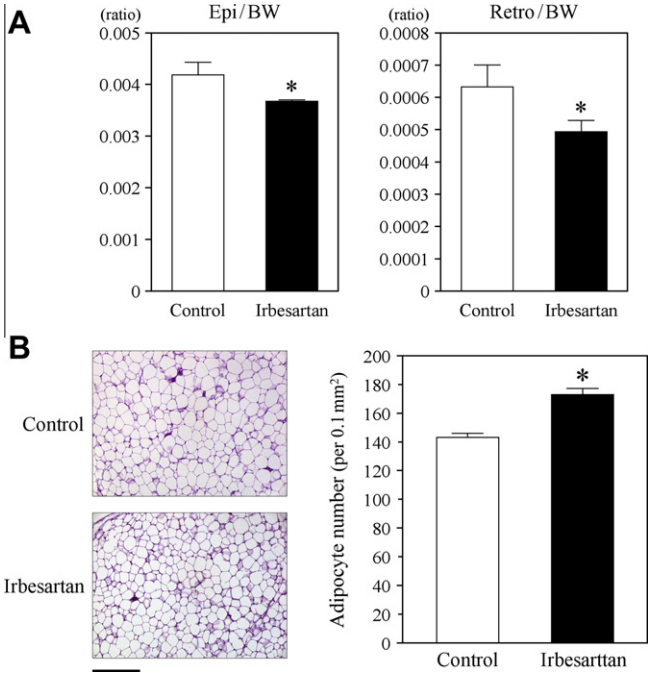

Fig. 1. Changes in adipose tissue weight and adipocyte number in white adipose tissue of atherosclerotic apolipoprotein E-deficient (ApoEKO) mice. White adipose tissue was taken after treatment of ApoEKO mice with irbesartan (50 mg/kg/day) for 4 weeks. (A) Ratio of adipose tissue weight to body weight. (B) Representative photomicrograph of epididymal white adipose tissue and bar graph of adipocyte number (density). Scale bar: 100 μm. $n = 7–8$ for each group. Epi: epididymal white adipose tissue. Retro: retroperitoneal white adipose tissue. BW: body weight. $*p < 0.05$ vs. control.

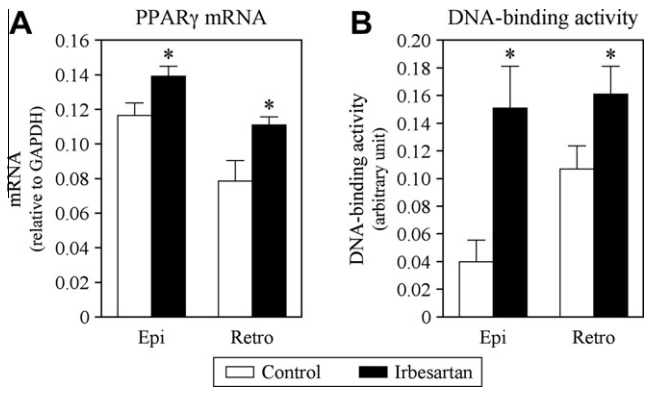

Fig. 2. Effect of irbesartan on mRNA expression and DNA-binding activity of PPARγ in vivo in white adipose tissue of atherosclerotic ApoEKO mice. White adipose tissue was taken as in Fig. 1. (A) Expression of PPARγ mRNA in white adipose tissue. (B) In vivo DNA-binding activity of PPARγ in white adipose tissue. $n = 7–8$ for each group. Epi: epididymal white adipose tissue. Retro: retroperitoneal white adipose tissue. $*p < 0.05$ vs. control.

3.3. Effect of irbesartan on expression of markers for insulin signaling in white adipose tissue

Treatment with irbesartan lowered plasma glucose level, while plasma insulin level was not changed (Fig. 3A). Of the markers for insulin signaling, expression of insulin receptor was increased in retroperitoneal adipose tissue (Fig. 3B). Expression of IRS-1 and GLUT4 tended to increase after treatment with irbesartan (Fig. 3C).

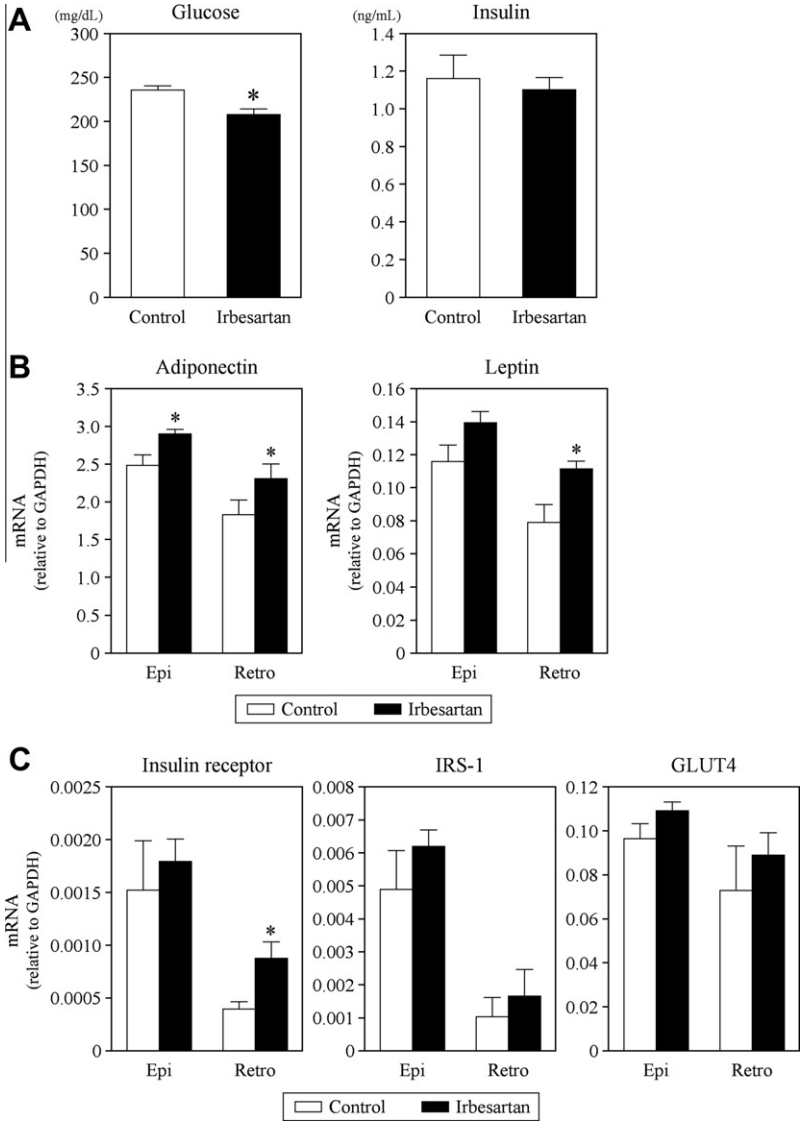

Fig. 3. Effect of irbesartan on plasma levels of glucose and insulin, and mRNA expression of adiponectin, leptin and markers for insulin signaling in white adipose tissue of atherosclerotic ApoEKO mice. White adipose tissue was taken as in Fig. 1. (A) Plasma levels of glucose and insulin without food restriction. (B) Expression of mRNA for adiponectin and leptin in white adipose tissue. (C) Expression of mRNA for insulin receptor, IRS-1 and glucose transporter type 4 (GLUT4) in white adipose tissue. $n = 7–8$ for each group. Epi: epididymal white adipose tissue. Retro: retroperitoneal white adipose tissue. $*p < 0.05$ vs. control.

4. Discussion

The present study showed that administration of an ARB, irbesartan, increased DNA-binding activity of PPARγ in white adipose tissue of atherosclerotic mice in vivo. In these mice, irbesartan decreased white adipose tissue weight and increased adipocyte number (or density), suggesting that irbesartan increased the number of small size adipocytes. Irbesartan also increased mRNA expression of PPARγ in white adipose tissue. It has been hypothesized that a PPARγ agonist induces adipocyte differentiation, thereby, causing smaller size of adipocytes and increasing insulin sensitivity [1]. Therefore, our results suggest that irbesartan improved adipose tissue dysfunction through an increase in PPARγ activity in white adipose tissue.

Metabolic syndrome is considered to be closely related to atherosclerosis and other cardiovascular diseases [10,11]. We have previously reported that ApoEKO mice, an atherosclerotic model, showed an increase in adipose tissue weight and enlargement of adipocyte size, compared with wild type mice [8]. In their white adipose tissue, the expression of PPARγ and transcription factors for adipocyte differentiation was also decreased [8]. Since PPARγ activation is considered to induce adipocyte differentiation, these results suggest that adipose tissue dysfunction in ApoEKO mice might be related to, at least in part, low activity of PPARγ in white adipose tissue.

Angiotensin II plays an important role not only in cardiovascular disease but also in metabolic disorders, such as diabetes and hyperlipidemia, mainly through AT_1 receptor-mediated signaling [12,13]. We have previously reported that AT_1 receptor deficiency caused an increase in PPARγ mRNA level in white adipose tissue and a decrease in adipocyte size and adipose tissue weight in atherosclerotic mice [8]. We also reported that an ARB induced similar changes in adipose tissue of type 2 diabetic model mice to those cause by AT_1 receptor deficiency [7,14]. However, the effect of AT_1 receptor blockade on in vivo tissue PPARγ activity in white adipose tissue is not yet clear.

Some ARBs are reported to act as an agonist for PPARγ [5]. Irbesartan is included in these ARBs and is expected to have actions to

improve metabolic syndrome. In the present study, we observed that treatment of ApoEKO mice with irbesartan increased not only the expression of PPARγ but also the DNA-binding activity of PPARγ in the nuclear fraction directly prepared from white adipose tissue (Fig. 2). In this adipose tissue in the irbesartan group, adipocyte number was higher and adipose tissue weight was lower than those in the control group (Fig. 1). Since the dose of irbesartan used in the present study did not affect systolic blood pressure and feeding, the changes in white adipose tissue did not seem to be caused by indirect actions of blood pressure lowering and/or calorie intake.

In our study, irbesartan seemed to increase insulin sensitivity in white adipose tissue by enhancing insulin receptor expression. According to the previous hypothesis that adipocyte differentiation improves adipocyte size and insulin resistance [1], the effect of irbesartan on insulin sensitivity in white adipose tissue may be secondary to activation of adipocyte differentiation following PPARγ activation. However, since we have previously reported that treatment with ARB improved insulin resistance in diabetic mice [8,14], it may be also possible that irbesartan affects insulin sensitivity in adipose tissue independently of PPARγ activation.

In summary, irbesartan induced in vivo activation of PPARγ in white adipose tissue of atherosclerotic mice. This action of irbesartan might be closely related to adipocyte differentiation and the improvement of adipose tissue function associated with atherosclerosis.

5. Conflict of interest

We have no conflict of interest to disclose.

Acknowledgment

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