original article

Azilsartan treatment improves insulin sensitivity in obese spontaneously hypertensive Koletsky rats

M. Zhao, Y. Li, J. Wang, K. Ebihara, X. Rong, K. Hosoda, T. Tomita & K. Nakao

Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan

Aim: Hypertension often coexists with insulin resistance. However, most metabolic effects of the antihypertensive agents have been investigated in nomotensive animals, in which different conclusions may arise. We investigated the metabolic effects of the new angiotensin II type 1 receptor blocker azilsartan using the obese Koletsky rats superimposed on the background of the spontaneously hypertensive rats.
Methods: Male Koletsky rats were treated with azilsartan (2 mg/kg/day) over 3 weeks. Blood pressure was measured by tail-cuff. Blood biochemical and hormonal parameters were determined by enzymatic or ELISA methods. Gene expression was assessed by RT-PCR.
Results: In Koletsky rats, azilsartan treatment lowered blood pressure, basal plasma insulin concentration and the homeostasis model assessment of insulin resistance index, and inhibited over-increase of plasma glucose and insulin concentrations during oral glucose tolerance test. These effects were accompanied by decreases in both food intake and body weight (BW) increase. Although two treatments showed the same effect on BW gain, insulin sensitivity was higher after azilsartan treatment than pair-feeding. Azilsartan neither affected plasma concentrations of triglyceride and free fatty acids, nor increased adipose mRNA levels of peroxisome proliferator-activated receptor (PPAR)γ and its target genes such as adiponectin, aP2. In addition, azilsartan downregulated 11β-hydroxysteroid dehydrogenase type 1 expression.
Conclusions: These results show the insulin-sensitizing effect of azilsartan in obese Koletsky rats. This effect is independent of decreases in food intake and BW increase or of the activation of adipose PPARγ. Our findings indicate the possible usefulness of azilsartan in the treatment of metabolic syndrome.
Keywords: angiotensin II type 1 receptor, azilsartan, insulin, obesity, peroxisome proliferator-activated receptor

Date submitted 25 April 2011; date of first decision 25 May 2011; date of final acceptance 7 July 2011

Introduction

Hypertension is one component of the metabolic syndrome. Hypertension often coexists with insulin resistance, glucose intolerance and hyperlipidaemia. It has been well documented that activation of the renin–angiotensin system is a common feature in patients with the metabolic syndrome [1]. Blockade of the renin–angiotensin system/angiotensin II type 1 receptor (AT1) signalling has been shown in clinical and experimental studies to improve the metabolic syndrome [1]. Some AT1 blockers (angiotensin II type 1 receptor blockers ARBs) have been shown to improve insulin sensitivity in rodents and humans [2–6]. However, most studies of the antihypertensive agents have been performed in nomotensive animals, in which different conclusions from those in hypertensive objectives may arise.

Azilsartan (TAK-536) is a new ARB. A randomized, double-blind, placebo-controlled trial shows that, in patients with stages 1 and 2 hypertension, azilsartan at its maximal dose has superior efficacy to both olmesartan and valsartan at their maximal, approved doses without increasing adverse events, providing higher rates of hypertension control within the ARB

class [7]. Radioligand binding and functional studies *in vitro* have shown that azilsartan inhibits human AT1 more potently than olmesartan, telmisartan, valsartan and irbesartan [8]. In nomotensive KK-A^y mice azilsartan has been found to be more effective than candesartan; it reduced plasma concentrations of glucose and fatty acids, decreased adipose tissue weight and adipocyte size and increased adipose expression of peroxisome proliferator-activated receptor (PPAR)γ and its target genes adiponectin and aP2; but did not affect blood pressure and plasma insulin concentrations [9]. The metabolic effects of the antihypertensive agent need to be further investigated in hypertensive objectives.

The Koletsky (fa^k/fa^k) rat carries a nonsense mutation in the leptin receptor and exhibits hyperphagia, obesity, hyperinsulinaemia/insulin resistance and hyperlipidaemia which are superimposed on the spontaneously hypertensive rat (SHR) background [6,10]. In the present study, we investigated the effects of azilsartan on metabolic abnormalities in the obese Koletsky rats.

Methods

Animals, Diet and Experimental Protocol

All animal procedures were in accordance with the 'Principles of laboratory animal care' (http://grants1.nih.gov/grants/olaw/

Correspondence to: Dr. Yuhao Li, Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan.
E-mail: yuhao@kuhp.kyoto-u.ac.jp; yuhao@pharm.usyd.edu.au

references/phspol.htm) and were approved by the Animal Ethics Committee, Kyoto University, Japan.

Male Wistar-Kyoto (WKY) rats, obese Koletsky (fa^k/fa^k) rats and their lean (+/+) littermates (SHR) were generous gifts from Japan SLC, Inc., Shizuoka, Japan. Rats were housed in a temperature controlled facility ($21 \pm 1°C$, $55 \pm 5\%$ relative humidity) with a 12-h light/dark cycle (two to three rats per cage). Animals were allowed free access to water and the standard diet (CLEA, Tokyo, Japan) for at least 1 week prior to starting the experiments at 10 weeks of age.

Experiment 1: Optimization of Azilsartan Dosage in Obese Koletsky Rats. Age-matched WKY rats and SHR were used as controls (n = 6 per group). As many as 24 obese Koletsky rats were divided into 4 groups (n = 6 per group): Koletsky azilsartan 0 mg/kg (control), Koletsky azilsartan 1 mg/kg, Koletsky azilsartan 2 mg/kg and Koletsky azilsartan 3 mg/kg. There were no differences in body weight (BW) among the Koletsky groups before treatments commenced. Animals in azilsartan-treated groups were administered azilsartan (1, 2 and 3 mg/kg, a generous gift from Takeda Co., Ltd., Japan, suspended in 5% Gum Arabic) by oral gavage once daily (9:00–10:00 hours) for 5 days. The WKY rats, SHR and Koletsky rats in control group received vehicle (5% Gum Arabic) alone. Rats were weighed once every 3–4 days to determine gavage volumes. To minimize the influence of measurement manipulation-induced stress on metabolic effects determined 2–3 weeks after treatments commenced in experiments 2 and 3, systolic blood pressure (SBP) was measured on day 5.

Experiment 2: Study of Metabolic Effects in Obese Koletsky Rats. In this experiment, 16 obese Koletsky rats were divided into two groups (n = 8 per group): Koletsky control (azilsartan 0 mg/kg) and Koletsky azilsartan (azilsartan 2 mg/kg). Eight lean (+/+) littermates (SHR) were used as controls. BWs were comparable between the Koletsky groups before treatments commenced. Animals in azilsartan group were administered azilsartan 2 mg/kg by oral gavage once daily (9:00–10:00 hours) for 21 days. The SHR and Koletsky control rats received vehicle (5% Gum Arabic) alone. Rats had free access to standard laboratory chow. Food intake was monitored daily and rats were weighed once every 3–4 days to determine gavage volume. SBP was measured on day 5 and blood samples were collected by retroorbital venous puncture under ether anaesthesia after rats had been fasted for 6 h on day 14, for determination of fasted plasma glucose, insulin, corticosterone, triglyceride and non-esterified fatty acids (NEFA) concentrations. Insulin sensitivity was expressed as the homeostasis model assessment of insulin resistance (HOMA-IR) index {[fasted insulin (μIU/ml) × fasted glucose (mM)]/22.5} [6,11,12]. Oral glucose tolerance tests (OGTT) were performed on day 17. Animals were weighed on day 21 and then killed by prompt dislocation of the neck vertebra. Epididymal white adipose tissues (eWAT) were collected and weighed. Segments of eWAT were snap frozen in liquid nitrogen and stored at −80°C for subsequent determination of gene analysis.

Experiment 3: Comparison of the Metabolic Effects of Azilsartan and Pair-feeding Treatments in Obese Koletsky Rats. In this experiment, 10 obese Koletsky rats were divided into two groups (n = 5 per group): Koletsky azilsartan (azilsartan 2 mg/kg) and Koletsky pair-feeding (azilsartan 0 mg/kg). BWs were insignificantly different between the groups before treatments commenced. Rats in azilsartan group had free access to standard laboratory chow. The food consumption in the pair-feeding group was restricted to that in azilsartan-treated animals (chow was provided daily at 10:00 hours). The other procedures were the same as that described in experiment 2.

SBP Measurement

SBP was measured 2–6 h after administration of azilsartan or vehicle in conscious rats by a tail-cuff method (MK-2000ST; Muromachi Kikai Co Ltd, Tokyo, Japan). At least six readings were taken for each measurement.

OGTT

To reduce the difference in plasma insulin concentrations between groups, rats were fasted for 15 h with free access to water before OGTT. Rats received a glucose solution (4 g/kg in 10 ml) by the oral route. Blood samples were collected prior to and 20, 60 and 120 min after administration of glucose solution for determination of plasma glucose and insulin concentrations.

Blood Biochemical Determination

Plasma concentrations of glucose, triglyceride and NEFA were determined using commercial enzymatic methods (kits from Wako, Osaka, Japan). Plasma insulin (kit from Morinaga, Tokyo, Japan) and corticosterone (kit from Cayman Chemical, Ann Arbor, MI, USA) concentrations were determined by ELISA.

Histological Examination

A portion of eWAT was fixed with 10% formalin and embedded in paraffin. Ten micron sections were cut and stained with haematoxylin and eosin for examination of adipose tissue histology (IX-81, Olympus Corporation, Tokyo, Japan). The adipocyte cross-sectional area was measured using an ImageJ 1.43 analysing system.

Gene Expression Analysis

Total RNA was isolated from eWAT of individual rats using TRIzol (Invitrogen, Osaka, Japan). Single-stranded cDNA was synthesized from 1 μg of total RNA using SuperScript III First-Strand Synthesis System for RT-PCR, according to the manufacturer's instructions (Invitrogen, Osaka, Japan). Quantitative RT-PCR was performed with an AB 7300 RT-PCR System using TaqMan (Applied Biosystems, Foster City, CA, USA). The sequences of primers and probes (Sigma-Genosys, Japan) used in the present study were as follows: PPARγ: sense, TGACCAGGGAGTTCCTCAAAA; antisense, AGCAAACTCAAACTTAGGCTCCAT; probe, CC

TGCGGAAGCCCTTTGGTGACT; adiponectin: sense, GGAC
CAAGAACACCTGCGTCT; antisense, TCCTGGTCACAATG
GGATACC; probe, TTCTCTCCAGGAGTGCCATCTCTGCC;
aP2: sense, TCCAGTGAGAACTTCGATGATTACA; antisense,
GGCCATACCGGCCACTTT; probe, TGGGAGTTGGCTTCG
CCACCAG; 11β-hydroxysteroid dehydrogenase type 1 (11β-
HSD1): sense, 5′-GCTGAAACAGAGCAATGGCAG-3′; anti-
sense, 5′-GAACCCATCCAGAGCAAACTTG-3′; probe, TGG
CTGGGAAAATGACCCAACCTCTGA; 18s: sense, GCAATT
ATTCCCCATGAACGA; antisense, CAAAGGGCAGGGACT
TAATCAAC; probe, AATTCCCAGTAAGTGCGGGTCATAA
GCTTG. Rat mitochondrial subunit 18s rRNA was selected as
the endogenous control (housekeeping gene).

Data Analysis

All results are expressed as means ± s.e.m. Data obtained from
experiments 1 and 2 were analysed by one-way analysis of
variance (ANOVA). If a difference was detected (F-ratio), the
Student–Newman–Keuls test was performed to locate the
differences between groups. Data obtained from experiment
3 were analysed using the Student's t-test. $p < 0.05$ was
considered to be statistically significant.

Results

Optimization of Azilsartan Dosage in Obese Koletsky Rats

SHR and obese Koletsky rats appeared hypertensive compared
to normal WKY controls; but there was no significant difference
between SHR and obese Koletsky rats (figure 1). Azilsartan was
administered by oral gavage (0–3 mg/kg once daily for 5 days);
controls received vehicle alone. From the dose–response
relationship, azilsartan (2 mg/kg) decreased SBP in obese
Koletsky rats to that of normal rats, whereas the 3 mg/kg dose
elicited hypotension (figure 1). From these findings azilsartan
2 mg/kg was selected for subsequent experiments.

Effects of Azilsartan Treatment on Glucose Metabolism in Obese Koletsky Rats

In this experiment, azilsartan 2 mg/kg showed similar blood
pressure-lowering effect to that of above experiment (data not
shown). In fasted obese animals plasma glucose concentrations
were unchanged (figure 2A); however, plasma insulin con-
centrations (figure 2B) and the HOMA-IR index (figure 2C)
were over fivefold higher than those in SHR. Azilsartan treat-
ment did not affect basal plasma glucose concentrations, but
decreased plasma insulin concentrations. The decline in insulin
concentration also contributed to the observed decrease in the
HOMA-IR index. Moreover, in the OGTT assessments, azil-
sartan modulated the abnormal increase in plasma glucose and
insulin concentrations (figure 2D, E).

Effects of Azilsartan Treatment on Adiposity-associated Variables in Obese Koletsky Rats

The hyperphagia observed in obese Koletsky rats (an increase
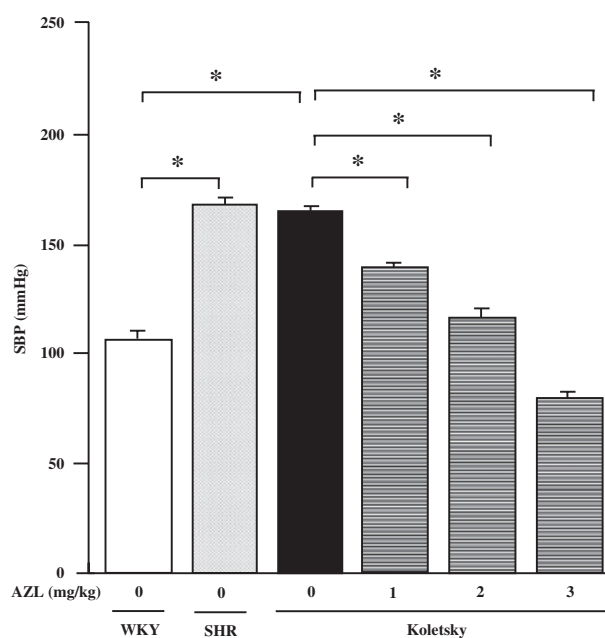in food intake of 34% over SHR control) was attenuated by

Figure 1. Systolic blood pressure (SBP) in male Wistar-Kyoto (WKY),
spontaneously hypertensive rats (SHR) and obese Koletsky rats. Animals
received azilsartan at different dosages or vehicle by oral gavage once daily.
SBP was measured with a tail-cuff method 2–5 h after the treatment on
day 5. Data are means ± s.e.m. (n = 6 each group). *$p < 0.05$ (analysis of
variance).

azilsartan treatment (figure 3A). Azilsartan also prevented the
excess BW increase that was evident in untreated obese Koletsky
rats (figure 3B). However, azilsartan treatment minimally
affected the increased eWAT weight (figure 3C) and adipocyte
size (figure 3D, E).

Effects of Azilsartan Treatment on Plasma Concentrations of Triglyceride, NEFA and Corticosterone in Obese Koletsky Rats

Compared to lean SHR-littermates, obese Koletsky rats
exhibited increased fasted plasma triglyceride concentration
(figure 4A), whereas NEFA concentration was unchanged
(figure 4B). Treatment of obese Koletsky rats with azilsartan
did not affect plasma concentrations of triglyceride or NEFA.

Plasma corticosterone concentrations in obese Koletsky rats
were over threefold higher than those in respective SHR control.
Azilsartan treatment minimally affected plasma corticosterone
concentrations (figure 4C).

Comparison of the Effects Between Azilsartan and Pair-feeding Treatments in Obese Koletsky Rats

The results in above experiments show that azilsartan at
2 mg/kg decreased food intake. To ensure that the actions
of azilsartan were independent of effects on food intake, a
comparison between azilsartan and pair-feeding experiments
was performed. The obese rats in two groups consumed similar
amounts of food (data not shown). In contrast with azilsartan
treatment, pair-feeding did not affect SBP (pair-feeding:
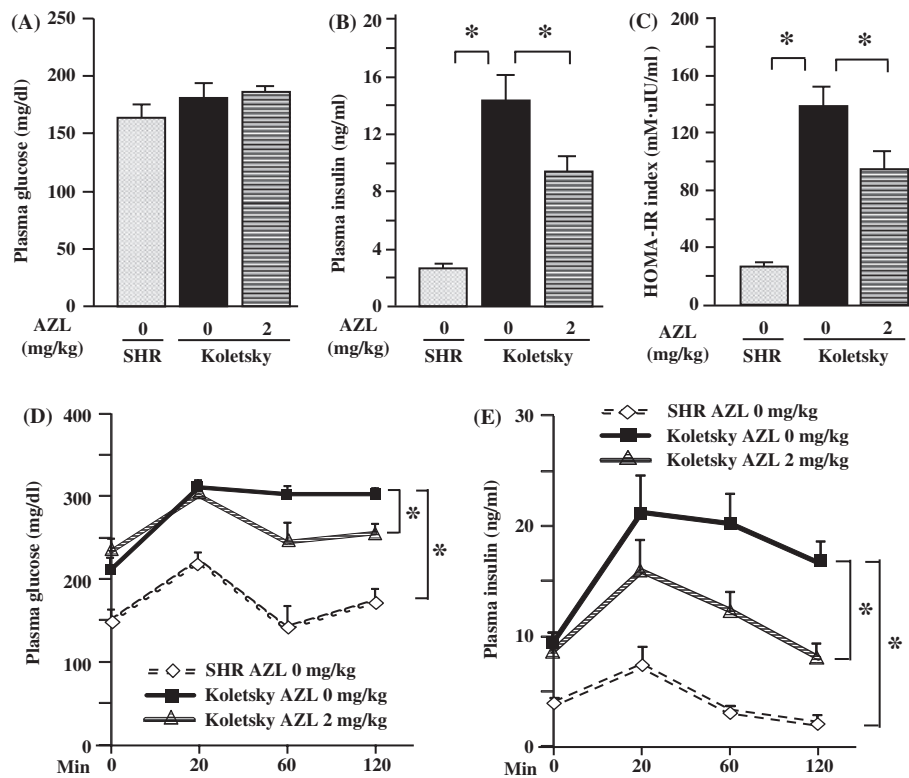175 ± 4 vs. azilsartan: 122 ± 2 mmHg, $p < 0.05$); there were

Figure 2. Fasted plasma glucose (A) and insulin (B) concentrations (day 14), the index of the homeostasis model assessment of insulin resistance (C), and glucose (D) and insulin (E) concentrations during oral glucose tolerance testing (day 17, glucose dosage: 4 g/kg) in male obese Koletsky rats and their littermates spontaneously hypertensive rat (SHR). Animals received azilsartan (2 mg/kg) or vehicle daily as described in the legend to figure 1. Data are means ± s.e.m. (n = 8 each group). *p < 0.05 (analysis of variance).

no differences in BW, eWAT weight and adipocyte size (data not shown). Although fasted plasma glucose concentrations (figure 5A) between two treatments were not different, fasted plasma insulin concentration (figure 5B), the HOMA-IR index (figure 5C) and plasma glucose concentrations during OGTT (figure 5D) were lower after treatment with azilsartan than pair-feeding.

Gene Expression Profile in Obese Koletsky Rats

In adipose tissue there was no significant difference in PPARγ, aP2 and 11β-HSD1 expression between two genotypes, while adiponectin mRNA level was decreased in obese Koletsky rats than SHR (p < 0.05, data not shown). Azilsartan treatment did not significantly affect PPARγ and adiponectin mRNA expression, but downregulated aP2 and 11β-HSD1 expression in obese Koletsky rats (p < 0.05, data not shown).

Discussion

The present findings clearly show that, in addition to its potent antihypertensive property, azilsartan treatment also improves insulin sensitivity in obese spontaneously hypertensive Koletsky rats. Azilsartan treatment decreased the hyperinsulinaemia, improved the HOMA-IR index and suppressed the over-increase in plasma glucose and insulin concentrations during OGTT. Thus, azilsartan treatment may diminish the risk

of cardiovascular morbidity and mortality as a result of hypertension, especially in the presence of insulin resistance and hyperinsulinaemia in obesity and type 2 diabetes.

As azilsartan treatment attenuated hyperphagia and decreased BW increase in obese Koletsky rats, we evaluated the impact of decreases in both food intake and BW gain on metabolic parameters. Although there was no difference in BW and adiposity variables between azilsartan and pair-feeding treatments, pair-feeding did not mimic the insulin-sensitizing effect of azilsartan. From these considerations it appears that azilsartan treatment improves insulin sensitivity via additional mechanisms.

Adipose PPARγ is the master regulator of adipogenesis and is activated by the thiazolidinediones that are used clinically to stimulate the action of insulin [13]. PPARγ-activating ligands alter fat topography and adipocyte phenotype and activate genes that regulate fatty acid metabolism and triglyceride storage [14]. Thus, the treatment with rosiglitazone decreased plasma concentrations of glucose, triglyceride and NEFA, but increased BW in obese Zucker rats [15–17]. Azilsartan (6.58 mg/kg/day delivered orally in chow) has been shown to decrease BW, adipose tissue weight and adipocyte size in KK-A[y] mice, which was accompanied by a decrease in NEFA concentrations, as well as enhanced adipose expression of PPARγ and its target genes adiponectin, aP2 [9]. In the present study, however, azilsartan minimally affected eWAT weight, adipocyte size and basal plasma concentrations
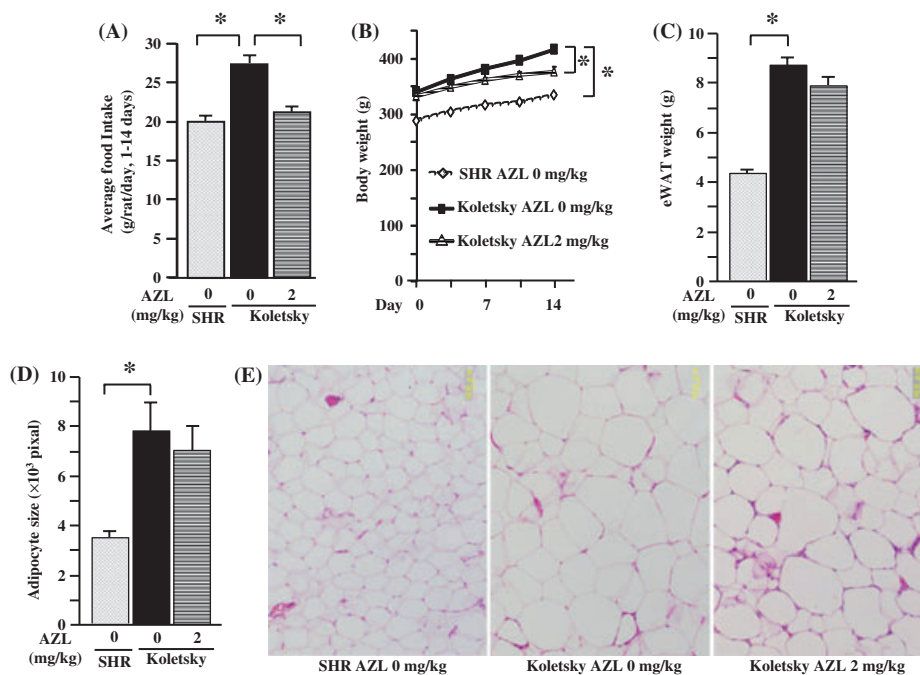
Figure 3. (A) Average food intake, (B) body weight, (C) epididymal white adipose tissue (eWAT) weight, (D) epididymal adipocyte size and (E) representative images showing histology of eWAT (haematoxylin and eosin staining, ×100) in male spontaneously hypertensive rat (SHR) and obese Koletsky rats. Animals received azilsartan (2 mg/kg) or vehicle daily for 21 days as described in the legend to figure 1. Food intake for 24 h was determined over a 14-day period with average food intake calculated accordingly. Data are means ± s.e.m. (n = 8 each group). *p < 0.05 (analysis of variance).
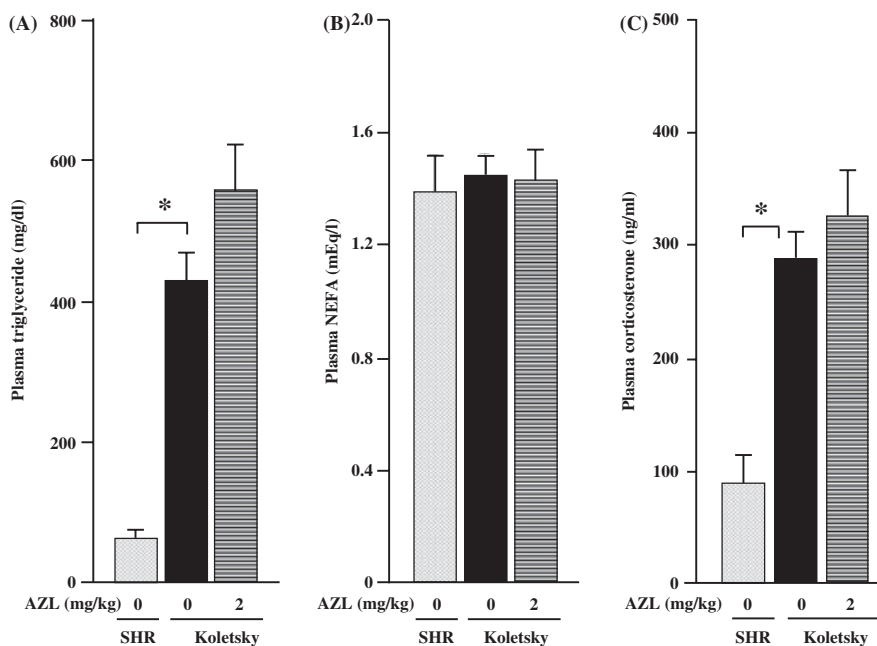

Figure 4. Fasted plasma concentrations of triglyceride (A), non-esterified fatty acids (NEFA) (B) and corticosterone (C) in male spontaneously hypertensive rat (SHR) and obese Koletsky rats on day 14. Animals received azilsartan (2 mg/kg) or vehicle daily as described in the legend to figure 1. Data are means ± s.e.m. (n = 8 each group).

of glucose, triglyceride and NEFA in obese Koletsky rats. Furthermore, azilsartan treatment did not upregulate PPARγ, adiponectin and aP2 expression in adipose tissue. Azilsartan at the concentrations up to 10^{-5} M did not show effect on PPARγ in the reporter gene assay in Chinese hamster ovary cells (data not shown). Thus, the effect of azilsartan on insulin sensitivity unlikely involves PPARγ activation in adipose tissue. Further investigations are now required to
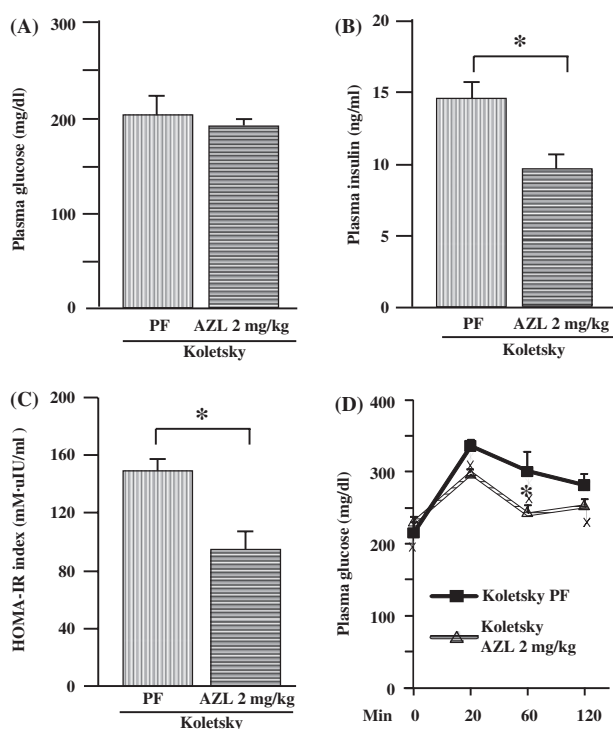
Figure 5. Fasted plasma glucose (A) and insulin (B) concentrations (day 14), the index of the homeostasis model assessment of insulin resistance (HOMA-IR) (C) and glucose concentrations (D) during oral glucose tolerance testing (day 17, glucose dosage: 4 g/kg) in male obese Koletsky rats. Animals received azilsartan (2 mg/kg) or pair-feeding and vehicle daily as described in section Methods. Data are means ± s.e.m. (n = 5 each group). *p < 0.05 (Student's t-test).

II interacts with ceramide production via its receptors, in which some cytokines, such as tumour necrosis factor-α, are involved [29]. In the present study, azilsartan treatment significantly downregulated expression of 11β-HSD1, as well as aP2 gene in adipose tissue of obese Koletsky rats. These results raise the possibility that azilsartan-elicited improvement of insulin sensitivity involves regulation of 11β-HSD1 activity. It needs to further investigate whether ceramide pathway is associated with azilsartan-elicited downregulation of adipose 11β-HSD1expression.

Considered together, the present results show that treatment with the new ARB azilsartan improves glucose metabolism in obese spontaneously hypertensive Koletsky rats. This insulin-sensitizing effect is independent of decreases in food intake and BW increase or of the activation of PPARγ in adipose tissue. Our findings indicate the possible usefulness of azilsartan in the treatment of the metabolic syndrome.

Acknowledgements

Conflict of Interest

The authors declared that they have no conflict of interest. M. Z. conducted data collection and contributed to writing the manuscript. Y. L. was involved in the design, analysis and writing of manuscript. J. W. conducted data collection and analysis. K. E., X. R., K. H., T. T., and K. N. contributed to the analysis.

assess whether the discrepancies between previous reports in mice and the present study conducted in obese rats are because of species differences (mouse vs. rat), blood pressure status (normotensive vs. hypertensive) and/or others. As azilsartan treatment showed minor effect on adipose tissue weight, significant attenuation of hepatomegaly, cardiac hypertrophy, renomegaly (our unpublished data) and/or others might mainly contribute to diminishment of BW gain after the treatment in obese Koletsky rats.

The adipose enzyme, 11β-HSD1, which locally converts inactive glucocorticoids into bioactive forms [18], plays an important role in the development of visceral obesity and its associated metabolic disturbances [19–22]. Recently, 11β-HSD1 has been recognized as a potential therapeutic target for the treatment of metabolic syndrome as various 11β-HSD1 inhibitors have been shown to improve insulin sensitivity in ob/ob, db/db, KK-A^Y and high fat diet-induced obese mice [23–26]. Differentiation of 3T3-L1 cells caused a strong increase in 11β-HSD1 protein levels; combination of dexamethasone and insulin induced 11β-HSD1 expression [27]. In contrast, an 11β-HSD1 inhibitor KR-66344 downregulated expression of 11β-HSD1 and adipogenesis-related genes in cortisone-treated 3T3-L1 cells [26]. Recently, we have shown that ceramide, an intracellular lipid second messenger, is a novel regulator of 11β-HSD1 expression and activity in cultured preadipocytes [28]. It is known that angiotensin

References

1. Prasad A, Quyyumi AA. Renin-angiotensin system and angiotensin receptor blockers in the metabolic syndrome. Circulation 2004; **110**: 1507–1512.

2. Benson SC, Pershadsingh HA, Ho CI et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 2004; **43**: 993–1002.

3. Negro R, Formoso G, Hassan H. The effects of irbesartan and telmisartan on metabolic parameters and blood pressure in obese, insulin resistant, hypertensive patients. J Endocrinol Invest 2006; **29**: 957–961.

4. Aksnes TA, Seljeflot I, Torjesen PA, Höieggen A, Moan A, Kjeldsen SE. Improved insulin sensitivity by the angiotensin II-receptor blocker losartan is not explained by adipokines, inflammatory markers, or whole blood viscosity. Metabolism 2007; **56**: 1470–1477.

5. Nishimura H, Sanaka T, Tanihata Y, Naito T, Higuchi C, Otsuka K. Losartan elevates the serum high-molecular weight-adiponectin isoform and concurrently improves insulin sensitivity in patients with impaired glucose metabolism. Hypertens Res 2008; **31**: 1611–1618.

6. Rong X, Li Y, Ebihara K et al. Irbesartan treatment up-regulates hepatic expression of PPARalpha and its target genes in obese Koletsky (fak/fak) rats: a link to amelioration of hypertriglyceridaemia. Br J Pharmacol 2010; **160**: 1796–1807.

7. White WB, Weber MA, Sica D et al. Effects of the Angiotensin receptor blocker azilsartan medoxomil versus olmesartan and valsartan on ambulatory and clinic blood pressure in patients with stages 1 and 2 hypertension. Hypertension 2011; **57**: 413–420.

8. Ojima M, Igata H, Tanaka M et al. In vitro antagonistic properties of a new angiotensin type 1 receptor blocker, azilsartan, in receptor binding and function studies. J Pharmacol Exp Ther 2010; **336**: 801–808.

9. Iwai M, Chen R, Imura Y, Horiuchi M. TAK-536, a new AT1 receptor blocker, improves glucose intolerance and adipocyte differentiation. Am J Hypertens 2007; **20**: 579–586.

10. Takaya K, Ogawa Y, Hiraoka J et al. Nonsense mutation of leptin receptor in the obese spontaneously hypertensive Koletsky rat. Nat Genet 1996; **14**: 130–131.

11. Rong X, Li Y, Ebihara K et al. An adipose tissue-independent insulin-sensitizing action of telmisartan: a study in lipodystrophic mice. J Pharmacol Exp Ther 2009; **331**: 1096–1103.

12. Rong X, Li Y, Ebihara K et al. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. Diabetologia 2010; **53**: 1727–1731.

13. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004; **10**: 355–361.

14. Sharma AM, Staels B. Peroxisome proliferator-activated receptor gamma and adipose tissue–understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab 2007; **92**: 386–395.

15. Cai XJ, Lister CA, Buckingham RE et al. Down-regulation of orexin gene expression by severe obesity in the rats: studies in Zucker fatty and Zucker diabetic fatty rats and effects of rosiglitazone. Brain Res Mol Brain Res 2000; **77**: 131–137.

16. Finegood DT, McArthur MD, Kojwang D et al. Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. Diabetes 2001; **50**: 1021–1029.

17. Reifel-Miller A, Otto K, Hawkins E et al. A peroxisome proliferator-activated receptor alpha/gamma dual agonist with a unique in vitro profile and potent glucose and lipid effects in rodent models of type 2 diabetes and dyslipidemia. Mol Endocrinol 2005; **19**: 1593–1605.

18. Seckl JR, Walker BR. Minireview: 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. Endocrinology 2001; **142**: 1371–1376.

19. Livingstone DE, Jones GC, Smith K et al. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 2000; **141**: 560–563.

20. Masuzaki H, Paterson J, Shinyama H et al. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001; **294**: 2166–2170.

21. Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi MC, Grino M. Expression of the mRNA coding for 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an in situ hybridization study. J Clin Endocrinol Metab 2002; **87**: 2701–2705.

22. Tomlinson JW, Walker EA, Bujalska IJ et al. 11β-Hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr Rev 2004; **25**: 831–866.

23. Alberts P, Engblom L, Edling N et al. Selective inhibition of 11beta-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. Diabetologia 2002; **45**: 1528–1532.

24. Alberts P, Nilsson C, Selen G et al. Selective inhibition of 11 beta-hydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycemic mice strains. Endocrinology 2003; **144**: 4755–4762.

25. Wang SJ, Birtles S, de Schoolmeester J et al. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 reduces food intake and weight gain but maintains energy expenditure in diet-induced obese mice. Diabetologia 2006; **49**: 1333–1337.

26. Park JS, Rhee SD, Kang NS et al. Anti-diabetic and anti-adipogenic effects of a novel selective 11β-hydroxysteroid dehydrogenase type 1 inhibitor, 2-(3-benzoyl)-4-hydroxy-1,1-dioxo-2H-1,2-benzothiazine-2-yl-1-phenylethanone (KR-66344). Biochem Pharmacol 2011; **81**: 1028–1035.

27. Kim J, Temple KA, Jones SA, Meredith KN, Basko JL, Brady MJ. Differential modulation of 3T3-L1 adipogenesis mediated by 11beta-hydroxysteroid dehydrogenase-1 levels. J Biol Chem 2007; **282**: 11038–11046.

28. Arai N, Masuzaki H, Tanaka T et al. Ceramide and adenosine 5′-monophosphate-activated protein kinase are two novel regulators of 11beta-hydroxysteroid dehydrogenase type 1 expression and activity in cultured preadipocytes. Endocrinology 2007; **148**: 5268–5277.

29. Berry C, Touyz R, Dominiczak AF, Webb RC, Johns DG. Angiotensin receptors: signaling, vascular pathophysiology, and interactions with ceramide. Am J Physiol Heart Circ Physiol 2001; **281**: H2337–2365.