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Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue

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Adipose tissue is recognized as a pivotal organ in the development of insulin resistance. This study seeks to determine the effect of angiotensin receptor blockade (ARB) on insulin resistance of adipocytes in culture and in a rat model of type 2 diabetes. Treatment of Otsuka Long-Evans Tokushima Fatty rats with the ARB L158809 for six months significantly lowered fasting plasma glucose, cholesterol and triglyceride levels but led to higher plasma adiponectin levels. Insulin resistance, measured by an intraperitoneal glucose tolerance test, of the treated rats was significantly improved along with an increase in the number of small differentiated adipocytes; however, epididymal fat mass decreased. Treatment significantly lowered lipid peroxidation and MCP-1 expression while increasing adiponectin production by the adipose tissue. ARB treatment significantly improved insulin sensitivity and markedly suppressed AT2-induced oxidative stress, PAI-1 and MCP-1 levels and NF-κB activation of adipocytes in culture. Treatment increased adiponectin and PPARγ expression along with intracellular triglyceride levels reflecting differentiation of the cultured adipocytes. Our study suggests that ARB treatment improves insulin resistance by modification of adipose tissue thereby blunting the development of diabetes.

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Recent hypertension trials have reported a lower incidence of diabetes mellitus among patients treated with renin-angiotensin system (RAS) inhibitor when compared with other classes of antihypertensive medications.[1–4] Although these studies were mostly performed with patients with hypertension or congestive heart failure, a 22% relative risk reduction for new-onset diabetes mellitus was demonstrated in a meta-analysis of randomized controlled trials, including 75,677 nondiabetic subjects after a mean follow-up period of 4–5 years.[5] Furthermore, the activity of RAS is also linked with the metabolic syndrome.[6] Taken together, these findings suggest that RAS may have an important role in the development of diabetes mellitus.

Adipocytes have recently attracted attention as dynamic endocrine cells that produce and secrete various bioactive molecules that are collectively referred to as adipocytokines, some of which affect the glucose homeostasis and insulin resistance of other tissues.[7–9] Adipose tissue has now been targeted as a pivotal organ for the development of insulin resistance, rather than for its energy storage function. Among the many adipocytokines, tumor necrosis factor-α (TNF-α), leptin, plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), resistin, visfatin, monocyte chemoattractant peptide-1 (MCP-1), and adiponectin have been implicated as active molecules in the development of insulin resistance.[7–11]

Interestingly, mature adipocytes express all components of the renin–angiotensin system, including angiotensinogen, angiotensin-converting enzyme, as well as angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors.[12] For these reasons, it can be hypothesized that the antidiabetic effect of RAS inhibition may derive from the effect of these drugs on adipocyte function, including the modulation of adipocytokines.

The primary objective of this study was to investigate the effect of an AT1 receptor antagonist, L158809, on insulin resistance under the hypothesis that angiotensin receptor blockade (ARB) may improve insulin resistance through the modulation of adipose tissues in Otsuka Long–Evans Tokushima Fatty (OLETF) rats. Additionally, to define the direct molecular mechanism of ARB, the effects of angiotensin

and ARB on adipocyte function and the synthesis of adipocytokines were evaluated in cultured murine adipocytes.

RESULTS

Biochemical parameters in experimental animals

Table 1 compares various biochemical parameters among three experimental groups of rats. The fasting plasma glucose level was significantly higher in OLETF rats compared with that in Long–Evans Tokushima Fatty (LETO) rats. Treatment of OLETF rats with L158809 for 6 months induced a significantly decreased fasting plasma glucose level. Body weight and kidney/body weight were also higher in OLETF rats than in LETO rats. L158809 treatment did not induce a significant change in body weight or kidney/body weight. Plasma insulin and c-peptide levels were significantly higher in OLETF rats than in LETO rats, although the L158809-treated group did not show a significant difference compared with the OLETF rats. Although serum creatinine levels and urine volumes were not different among the three groups, urinary albumin excretion in the OLETF rats was significantly higher than in the LETO rats. L158809 treatment significantly reduced urinary albumin excretion. Systolic blood pressure was greater in OLETF rats than in LETO rats, and L158809 treatment induced a significant decrease in systolic blood pressure.

Effect of L158809 on insulin resistance and metabolic parameters in experimental animals

As shown in Figure 1, improved glucose intolerance after treatment with L158809 in OLETF rats was confirmed by an i.p. glucose tolerance test (GTT). Although the basal levels of fasting blood glucose were slightly lower in the L158809-treated group, GTT clearly demonstrated improved glucose intolerance in L158809-treated rats vs OLETF rats. In accordance with improved glucose intolerance, L158809 treatment significantly decreased plasma total cholesterol and triglyceride levels (cholesterol, LETO; 113 ± 7.9, OLETF; 147 ± 23, OLETF + L158809; 100 ± 14, triglyceride, LETO; 39 ± 11, OLETF; 167 ± 19, OLETF + L158809; 69 ± 21, $P < 0.05$) (Figure 2a). In addition, plasma adiponectin levels were decreased in OLETF rats compared with control LETO rats, and L158809 treatment induced a significant increase in plasma adiponectin levels (LETO; 11 ± 1.2, OLETF; 8.1 ± 0.2, OLETF + L158809; 9.7 ± 0.36, $P < 0.05$) (Figure 2b). However, the homeostasis model assessment indices (HOMA-IRs) did not show a significant difference among the three groups (LETO; 0.65 ± 0.12, OLETF; 0.77 ± 0.11, OLETF + L158809; 0.69 ± 0.21) (Figure 2c).

Table 1 | Biochemical parameters in experimental animals

	LETO	OLETF	OLETF+L158809
Body weight (g)	516.6 ± 28.8	640.0 ± 22.4*	591.7 ± 58.4*
Kidney/BW/10^3	0.33 ± 0.05	0.36 ± 0.01*	0.38 ± 0.03*
FPG (mg/100 ml)	108 ± 11.2	134.7 ± 20**	126.3 ± 8.7*,#
P-insulin (ng/ml)	1.32 ± 0.24	2.48 ± 0.45*	2.40 ± 0.21*
P-c-peptide (pM)	655 ± 57	4754 ± 566**	4495 ± 612**
P-Cr (mg/100 ml)	0.64 ± 0.04	0.60 ± 0.11	0.59 ± 0.17
UV (ml/day)	17.6 ± 10.5	16.1 ± 4.43	17.5 ± 4.7
UAE (μg/mgCr/day)	2.48 ± 1.5	144.3 ± 22.8***	49.7 ± 11.4**,##
UAE (μg/day)	12.1 ± 3.4	443.3 ± 28.4***	116.5 ± 21.8**,##
SBP (mm Hg)	120.5 ± 18.4	146.5 ± 19.2**	119.8 ± 17.7#
Kid-Chol (mg/100 g)	273 ± 37	486 ± 65*	322 ± 46#
Kid-TG (mg/100 g)	186 ± 23	273 ± 33*	201 ± 27#
U-isopros (ng/day)	47.3 ± 11.2	112.4 ± 21.2*	67.3 ± 18.9#
Fat-lipid hydroperoxide (nmol/mg)	12.3 ± 2.33	31.8 ± 11.9**	18.3 ± 3.1#

BW, body weight; fat-lipid hydroperoxide, lipid peroxidation (hydroperoxide) in adipose tissue (nmol per mg protein); FPG, fasting plasma glucose; Kid-Chol, total cholesterol content in kidney (mg/100λg tissue); Kid-TG, triglyceride content in kidney (mg/100λg tissue); P-c-peptide, plasma c-peptide concentration; P-Cr, plasma creatinine concentration; P-insulin, plasma insulin concentration; SBP, systolic blood pressure; UAE, urinary albumin excretion; U-isopros, 24λh urinary excretion of 8-isoprostane; UV, urine volume.
Values are expressed as mean ± s.e.m.
*$P < 0.05$, LETO vs OLETF and OLETF+L158809; **$P < 0.01$, LETO vs OLETF and OLETF+L158809; ***$P < 0.001$, LETO vs OLETF; #$P < 0.05$, OLETF vs OLETF+L158809; ##$P < 0.01$, OLETF vs OLETF+L158809.

Effect of L158809 on histological changes of kidney and adipose tissue in experimental animals

Figure 3 shows the representative renal pathology in the experimental groups at the end of the study period. Diabetic OLETF rats showed more severe glomerulosclerosis when compared with the LETO rats. Consistent with the marked attenuation of albuminuria, the glomerulosclerotic index was significantly improved in the L158809-treatment group. Next, this study assessed whether the differences in body weight were related to the alteration in adiposity and to the L158809-induced phenotypic changes in adipose tissue. As shown in Figure 4, adipose tissue obtained from epididymal fat revealed that OLETF rats had larger adipocytes than LETO rats, and L158809 treatment restored their phenotype to small differentiated adipocytes (Figure 4a–c). In addition to these phenotypic changes, epididymal fat mass in OLETF rats was also significantly higher than in LETO rats, and the L158809-treated group showed a significant decrease over OLETF rats (LETO; 0.54 ± 0.05, OLETF; 1.10 ± 0.15, OLETF + L158809; 0.71 ± 0.10, $P < 0.05$) (Figure 4d).

Effect of L158809 on renal lipid metabolism in experimental animals

As L158809 improves albuminuria and glomerulosclerosis associated with improvement in systemic metabolic abnormalities,

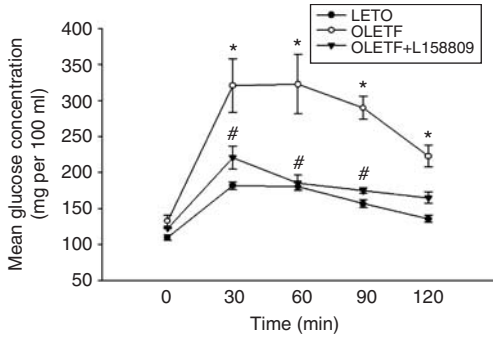

Figure 1 | Glucose tolerance test in experimental animals. L158809 group was treated for 6 months with L158809. Data are shown as the mean ± s.e.m. *$P < 0.05$, LETO vs OLETF; #$P < 0.05$, OLETF vs OLETF with L158809.