

A Rho-kinase inhibitor, fasudil, prevents development of diabetes and nephropathy in insulin-resistant diabetic rats

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Abstract

Fasudil, a Rho-kinase inhibitor, may improve insulin signaling. However, its long-term effect on metabolic abnormalities and its preventive effect on diabetic nephropathy are still unknown. We assessed these effects of fasudil in insulin-resistant diabetic rats, comparing them with those of an angiotensin II receptor blocker, olmesartan. Male Otsuka Long-Evans Tokushima fatty (OLETF) and Long-Evans Tokushima Otsuka, non-diabetic control, rats at 15 weeks of age were used. OLETF rats were randomized to receive a low or a high dose of fasudil or olmesartan for 25 weeks. To examine the therapeutic effects after the development of diabetes, OLETF rats at 30 weeks of age were given fasudil for 10 weeks. Administration of high-dose fasudil completely suppressed the development of diabetes, obesity, and dyslipidemia and increased serum adiponectin levels in OLETF rats. High-dose olmesartan also

decreased hemoglobin A1c and increased serum adiponectin. There was a significant correlation between hemoglobin A1c and serum adiponectin or free fatty acid levels. The treatment with high-dose fasudil ameliorated proteinuria, glomerulosclerosis, renal interstitial fibrosis, and macrophage infiltration in OLETF rats. Olmesartan, even at the low dose, suppressed renal complications. The treatment with fasudil after the development of diabetes improved the metabolic abnormalities in OLETF rats, but could not suppress the progression of nephropathy. We conclude that the long-term treatment with fasudil prevents the development of diabetes, at least in part, by improving adipocyte differentiation in insulin-resistant diabetic rats. Early use of fasudil may prevent diabetic nephropathy.

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Introduction

The population with type 2 diabetes mellitus and metabolic syndrome has been increasing worldwide and diabetic nephropathy is a leading cause in many countries, requiring renal replacement therapy. Obesity and insulin resistance are common characteristics in these conditions. To improve insulin resistance, biguanides and thiazolidinediones are widely used. Although these agents are very effective, serious adverse effects including lactic acidosis with biguanides, edema, body weight gain, heart failure, and liver dysfunction due to thiazolidinediones are well known. These facts indicate the need for a better therapeutic agent for type 2 diabetes.

A small GTP-binding protein, Rho, and its effector, Rho-kinase, have several physiological and pathological functions, including stress fiber formation, cytokinesis, and cell migration, and contribute to calcium ion sensitivity in smooth muscle cells (Fukata *et al.* 2001). Rho/Rho-kinase participates in the regulation of systemic vascular tone and abnormal contraction of vascular smooth muscle, inducing hypertension and vasospasm of the coronary and cerebral arteries. Y-27632, a Rho-kinase inhibitor, dramatically reduced blood pressure in several hypertensive rats (Uehata

et al. 1997). Another Rho-kinase inhibitor, fasudil, prolonged the maximum duration of exercise and time to the onset of ST segment depression in patients with stable effort angina (Shimokawa *et al.* 2002). In renal diseases, fasudil prevented hypertensive renal injuries by inhibiting expression of extracellular matrix genes, oxidative stress, cell proliferation, and macrophage infiltration in several hypertensive models (Kanda *et al.* 2003, Nishikimi *et al.* 2004, Ishikawa *et al.* 2006). Fasudil decreased urinary protein levels and reversed reduced nephrin expression in a puromycin aminonucleoside-induced nephrosis model (Shibata *et al.* 2006).

Insulin-stimulated GLUT4 translocation and glucose uptake in skeletal muscle are regulated by an insulin receptor/insulin receptor substrate (IRS)-1/phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Kanai *et al.* 1993). A selective resistance to this pathway was demonstrated in the vascular tissues of insulin-resistant obese Zucker rats (Jiang *et al.* 1999). In vascular smooth muscle cells, activation of RhoA/Rho-kinase, ROK- α , increased the association between Rho-kinase and IRS-1, leading to inhibition of insulin signaling (Begum *et al.* 2002). Recently, it was reported that treatment with fasudil for 4 weeks corrected the glucose and lipid metabolism in obese Zucker rats through

the improvement in insulin signaling in skeletal muscles (Kanda *et al.* 2006). This finding may allow a new therapeutic strategy for the treatment of metabolic syndrome and type 2 diabetes mellitus. However, the long-term effect of Rho-kinase inhibitors on metabolic abnormalities and its preventive effect on diabetic nephropathy are still unknown.

Based on these findings, we hypothesized that long-term administration of a Rho-kinase inhibitor might prevent the development and progression of diabetes and diabetic nephropathy in a spontaneous insulin-resistant diabetic model: Otsuka Long–Evans Tokushima fatty (OLETF) rats, which are obese, have hyperglycemia, hyperinsulinemia, hyperlipidemia, and hypertension (Kawano *et al.* 1992). First, fasudil was given to OLETF rats for 25 weeks from the early phase. Secondly, fasudil was given for 10 weeks after the development of diabetes to assess the therapeutic effects. In addition, these effects of fasudil were compared with those of an angiotensin II receptor blocker (ARB), for which the inhibition of the new development of diabetes (Lindholm *et al.* 2002, Julius *et al.* 2004, Yusuf *et al.* 2005) and renoprotective effects beyond lowering blood pressure (Brenner *et al.* 2001, Lewis *et al.* 2001, Viberti *et al.* 2002) have been proven in many clinical trials.

Materials and Methods

Materials

Male OLETF and Long–Evans Tokushima Otsuka (LETO) rats were obtained from Otsuka Pharmaceutical Tokushima Research Institute (Tokushima, Japan). Fasudil was obtained from Asahi Kasei Pharma Corporation (Tokyo, Japan) and olmesartan from Sankyo (Tokyo, Japan). The antibody against anti-rat ED1, which is a marker of monocytes/macrophages, was obtained from Cymbus Biotechnology (Chandlers Ford, Hants, UK); the antibody against phosphorylated myosin phosphatase target subunit (MYPT)-1, which is a marker of activation of the Rho/Rho-kinase pathway, from Upstate (Lake Placid, NY, USA); and the labeled streptavidin biotin (LSAB) kit from Dako (Carpinteria, CA, USA).

Experimental protocols

Protocol 1 Pathogen-free male OLETF and age-matched LETO, non-diabetic control, rats were used. Rats were given free access to tap water and standard rat chow. All studies followed the guidelines of National Defense Medical College for the care and use of laboratory animals in research. At 15 weeks of age, OLETF rats were divided into five groups: an untreated group, a low-dose fasudil (30 mg/kg) treatment group, a high-dose fasudil (100 mg/kg) treatment group, a low-dose olmesartan (5 mg/kg) treatment group, and a high-dose olmesartan (10 mg/kg) treatment group ($n=7$ in each), matched for body weight (average 551.5 ± 10.3 g) and blood glucose (average 136.0 ± 5.6 mg/dl). Fasudil and olmesartan

were dissolved in drinking water. These agents were given for 25 weeks. Laboratory data such as glucose, lipid and renal function, and renal pathological changes were compared among the six groups, including untreated LETO rats ($n=5$). Systolic blood pressure was measured by the tail-cuff method and urine samples over 24 h were collected in metabolic cages at 40 weeks of age. After anesthesia by i.p. injection with sodium pentobarbital (50 mg/kg), blood was collected from the aorta between 0900 and 1000 h under non-fasting conditions and the kidneys were removed. One kidney was rapidly frozen in liquid nitrogen, and the other kidney was fixed in 10% formalin and embedded in paraffin.

Protocol 2 At 30 weeks of age, ten OLETF rats were divided into two groups: an untreated group ($n=5$) and a high-dose fasudil (100 mg/kg) treatment group ($n=5$), matched for body weight (703.6 ± 12.4 vs 691.4 ± 29.8 g; $P=0.72$) and blood glucose (293.6 ± 35.1 vs 296.8 ± 46.0 mg/dl; $P=0.96$). Fasudil was given for 10 weeks. The same parameters as Protocol 1 were compared among the three groups, including untreated LETO rats ($n=5$). At the end of the study, the same procedures as Protocol 1 were performed.

Biochemical assays

Total blood glucose obtained from the tail vein was measured by the glucose oxidase method. Serum creatinine, total cholesterol, triglycerides, free fatty acid (FFA), and urinary creatinine and protein were measured by standard methods using an automatic analyzer, Hitachi 7180 (Hitachi). Creatinine clearance was calculated using the standard formula. Hemoglobin A1c levels were measured by the latex agglutination method. Serum adiponectin levels were determined by enzyme-linked immunosorbent assay (Otsuka, Tokyo, Japan).

Renal histological study

All formalin-fixed kidney sections (3 μ m) were stained with periodic acid-Schiff (PAS) and Masson's trichrome. Fifty full-sized glomeruli for each specimen were assessed on PAS-stained sections under a high-power field ($\times 400$) and the levels of glomerulosclerosis in each glomerulus were semiquantitatively scored as follows: 0, no sclerosis; 1, sclerosis $<10\%$ of glomeruli; 2, sclerosis 10–25% of glomeruli; 3, sclerosis 25–50% of glomeruli; 4, sclerosis $>50\%$ of glomeruli. To evaluate interstitial fibrosis, 20 fields for each section were assessed on Masson's trichrome-stained sections ($\times 200$). Semiquantitative analysis in each field was assessed as follows: 0, no fibrosis; 1, fibrosis $<10\%$ of areas; 2, fibrosis 10–25% of areas; 3, fibrosis 25–50% of areas; 4, fibrosis $>50\%$ of areas. The averages of the glomerulosclerosis and interstitial fibrosis scores were calculated from the total evaluated glomeruli or interstitial lesions in each section. These microscopic evaluations were performed by two observers without prior prejudicial information.

Immunohistochemistry

Three micrometer thick formalin-fixed sections were used to detect ED1-positive cells in the kidney. Deparaffinized sections were treated with proteinase K for 7 min for optimal

antigen retrieval and incubated with 3% H₂O₂ for 10 min to block endogenous peroxidase activity. After blocking with 7% bovine serum albumin in PBS, the sections were incubated with a primary antibody for ED1 (×200) for 60 min at 37 °C. Thereafter, the sections were incubated with

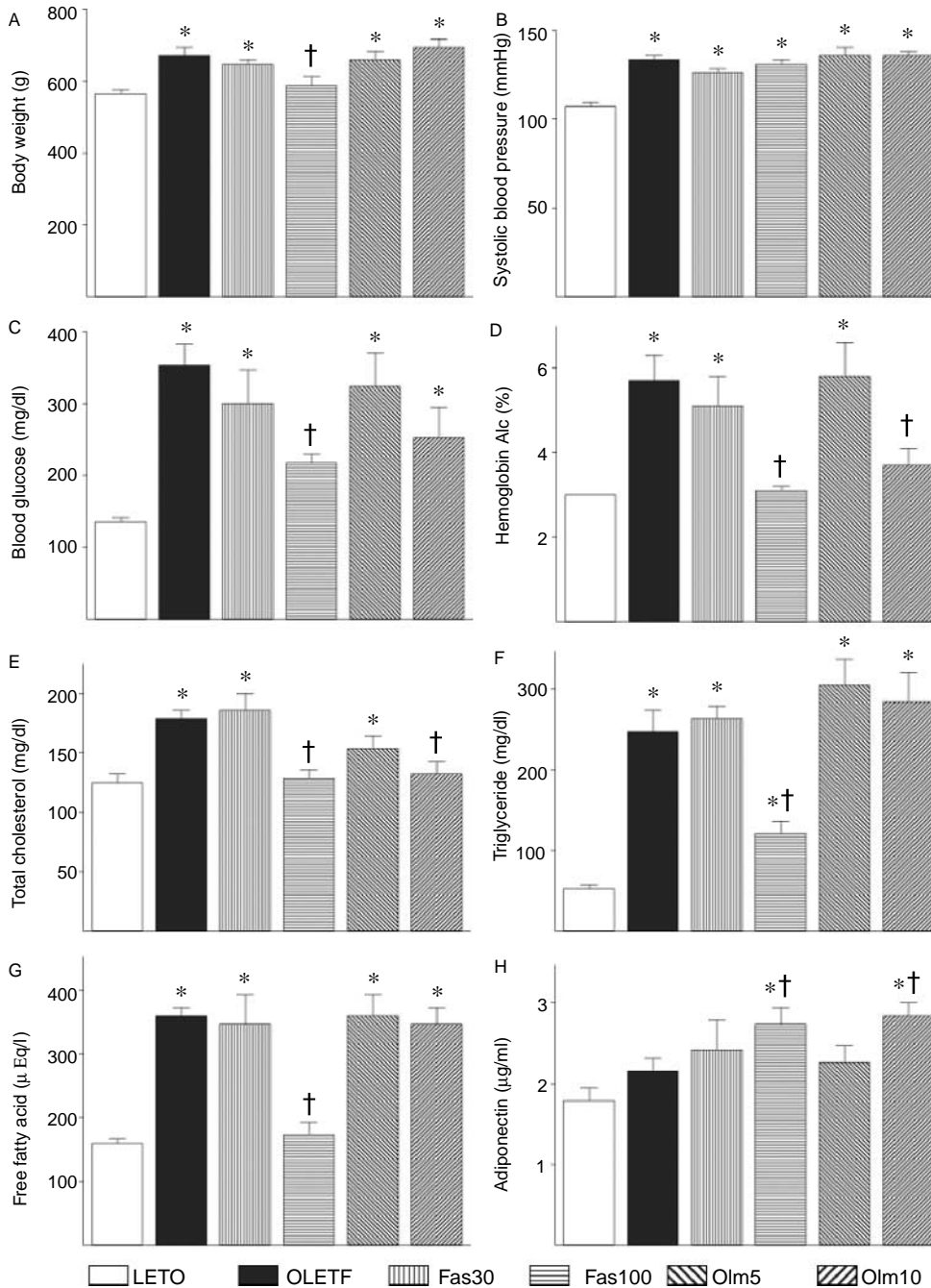


Figure 1 Body weight (A), blood pressure (B), glucose (C), hemoglobin A1c (D), and lipid metabolism (E–H) in OLETF rats treated with fasudil or olmesartan in Protocol 1 (n = 5–7). Fas30 or Fas100, OLETF rats treated with 30 or 100 mg/kg fasudil dissolved in drinking water; Olm5 or Olm10, OLETF rats treated with 5 or 10 mg/kg olmesartan dissolved in drinking water. *P < 0.05 vs LETO rats, †P < 0.05 versus untreated OLETF rats.

a DAKO LSAB system link antibody for 10 min, followed by incubation with peroxidase-conjugated streptavidin for 10 min at room temperature. After washing with PBS, the sections were stained with a 3,3'-diaminobenzidine solution and then counterstained with hematoxylin. More than 20 non-overlapping areas for each section were assessed under high-power magnification ($\times 400$), and the number of ED1-positive cells in each area was counted and averaged in each group.

Western blot analysis

To assess activation of Rho/Rho-kinase pathway in LETO and OLETF rats, western blot analysis of phosphorylated MYPT-1 was performed. The samples for western blotting were prepared from the renal cortex of LETO and OLETF rats. Protein was extracted from each renal sample by homogenizing it in a lysis buffer. The composition of the lysis buffer was 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 1% NP40, and a protease inhibitor cocktail (Sigma). Protein concentration was assayed by the Bio-Rad protein assay reagent, and 50 μ g protein were applied to each well. Samples were denatured for 3 min at 98 °C, electrophoresed on a gradient (4–15%) polyacrylamide gel (Bio-Rad Laboratories), and electrophoretically transferred onto nitrocellulose membranes. After blocking with a blocking buffer (Pierce, Rockford, IL, USA), the membrane was incubated with a primary antibody for phosphorylated MYPT-1 ($\times 500$) overnight at 4 °C. After washing with Tris-buffered saline containing 0.05% Tween 20, horseradish peroxidase-linked anti-rabbit IgG ($\times 2000$) was incubated for 1 h at room temperature. The specific protein expression was visualized using enhanced chemiluminescence western blotting detection reagents (Amersham). The phosphorylated MYPT-1 protein levels were corrected by the β -actin protein levels.

Statistical analysis

Results are expressed as the mean \pm S.E.M. Statistical analyses were performed using analysis of variance followed by Fisher's multiple comparisons. Correlations between several parameters were assessed by Spearman's correlation test. A *P* value of <0.05 was considered significant.

Results

Effects of fasudil and olmesartan on metabolic abnormalities in OLETF rats

The body weights of OLETF rats at 40 weeks of age were significantly higher than those of LETO rats (Fig. 1A). Systolic blood pressure in OLETF rats was higher than that of LETO rats (Fig. 1B). OLETF rats had significantly increased blood glucose, hemoglobin A1c, serum total cholesterol, triglycerides, and FFA levels compared with those of LETO rats (Fig. 1C–G). Serum adiponectin levels showed no significant difference between OLETF and LETO rats (Fig. 1H). Administration of low-dose fasudil did not affect these parameters in OLETF rats. The body weights of OLETF rats treated with high-dose fasudil were not different from those of LETO rats (Fig. 1A). High-dose fasudil did not change systolic blood pressure in OLETF rats (Fig. 1B), as observed in the previous reports (Nishikimi *et al.* 2004, Ishikawa *et al.* 2006). Administration of high-dose fasudil completely suppressed the development of diabetes and dyslipidemia in OLETF rats (Fig. 1C–G). Serum adiponectin levels in OLETF rats treated with high-dose fasudil were significantly higher compared with those in LETO or untreated OLETF rats (Fig. 1H). Olmesartan did not affect the body weights or blood pressure in OLETF rats (Fig. 1A and B). The treatment with high-dose olmesartan in OLETF rats significantly decreased hemoglobin A1c and total cholesterol levels, as well as increased serum adiponectin levels compared with untreated OLETF rats (Fig. 1D, E and H). However, high-dose olmesartan did not improve serum triglycerides or FFA levels (Fig. 1F and G).

In OLETF rats, serum adiponectin and FFA levels, which are associated with adipocyte differentiation, were significantly correlated with blood glucose, hemoglobin A1c, and total cholesterol levels (Table 1).

Phosphorylated MYPT-1 levels in the renal cortex of OLETF rats

Phosphorylated MYPT-1 protein levels in the renal cortex of OLETF rats at 40 weeks of age were significantly higher than those of LETO rats (Fig. 2). High-dose fasudil significantly suppressed the phosphorylated MYPT-1 levels in OLETF rats. Low-dose fasudil and olmesartan (both doses) did not affect the phosphorylated MYPT-1 levels.

Table 1 Correlation between adiponectin or free fatty acid and blood glucose, hemoglobin A1c, or lipids in OLETF rats

	Blood glucose	Hemoglobin A1c	Total cholesterol	Triglyceride	Free fatty acid
Adiponectin	$r = -0.748$ $P < 0.001$	$r = -0.822$ $P < 0.001$	$r = -0.594$ $P = 0.001$	NS	NS
Free fatty acid	$r = 0.544$ $P = 0.002$	$r = 0.528$ $P = 0.002$	$r = 0.589$ $P < 0.001$	$r = 0.561$ $P = 0.001$	–

NS; not significant.

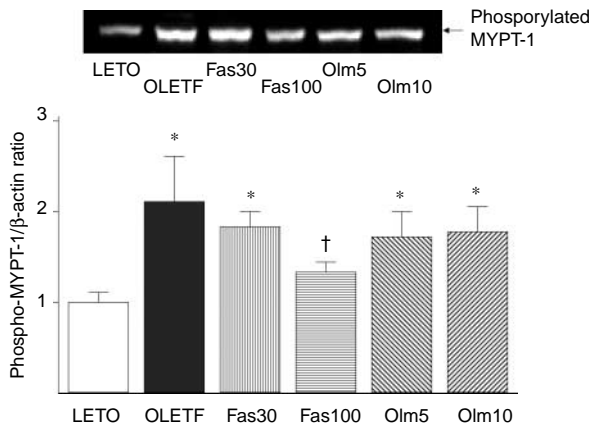


Figure 2 Phosphorylated MYPT-1 protein expression in the renal cortex of LETO and OLETF rats in Protocol 1. The photograph shows the representative results of western blotting for phosphorylated MYPT-1 (130 kDa). The bar graph shows the abundance of phosphorylated MYPT-1 protein expression relative to β-actin. * $P < 0.05$ vs LETO rats, † $P < 0.05$ vs untreated OLETF rats.

Effects of fasudil and olmesartan on renal complications in OLETF rats

Creatinine clearance did not show significant differences among the groups (Fig. 3A). Urinary protein levels of OLETF rats were significantly higher than those of LETO rats (Fig. 3B). High-dose fasudil and both doses of olmesartan significantly suppressed the excretion of urinary protein.

Glomerular lesions in OLETF rats were characterized by hyalinosis, thickening of the basement membrane, mesangial expansion, and sclerotic lesions (Fig. 4A). Glomerulosclerosis and the interstitial fibrosis score in OLETF rats were significantly higher than those of LETO rats (Fig. 4B and C). The number of ED1-positive cells in the kidneys was significantly increased in OLETF rats (Fig. 4D). These pathological changes and ED1-positive cell infiltration were suppressed by the treatments with high-dose fasudil and both doses of olmesartan (Fig. 4A–D). Low-dose fasudil also

suppressed the interstitial fibrosis score and the number of ED1-positive cells compared with untreated OLETF rats.

Effects of fasudil after the development of diabetes in OLETF rats

Administration of high-dose fasudil for 10 weeks after the development of diabetes did not affect the body weight (664.8 ± 41.5 vs 596.6 ± 42.4 g; $P = 0.19$) or the systolic blood pressure (138.5 ± 3.4 vs 141.1 ± 2.1 mmHg; $P = 0.54$) in OLETF rats. The blood glucose and hemoglobin A1c levels in OLETF rats treated with fasudil were significantly lower than those in untreated OLETF rats (Fig. 5A and B). With short-term treatment with fasudil, serum total cholesterol, triglycerides, and FFA also significantly decreased and serum adiponectin increased in OLETF rats (Fig. 5C–F). Urinary protein levels, glomerulosclerosis, and interstitial fibrosis scores did not change after treatment with fasudil (Fig. 6A–C).

Discussion

In this study, we demonstrated that administration of a Rho-kinase inhibitor, fasudil, prevented the development and progression of obesity, hyperglycemia, dyslipidemia, and nephropathy in diabetic OLETF rats. The improvement in blood glucose and hemoglobin A1c levels was strongly correlated with serum adiponectin and FFA levels in OLETF rats.

Adipose tissue, especially visceral fat, expresses numerous genes and secretes biologically active molecules, called ‘adipocytokines’ (Maeda *et al.* 1997, Funahashi *et al.* 1999). Among them, leptin, resistin, tumor necrosis factor (TNF)-α, and interleukin-6 are proinflammatory adipocytokines, while adiponectin is an anti-inflammatory adipocytokine. An imbalance in them leads to the development of atherosclerosis and insulin resistance (Axelsson *et al.* 2005). Since higher concentrations of adiponectin protected against later development of type 2 diabetes in humans (Lindsay *et al.* 2002), adiponectin has been thought to play a key role in the development of type 2 diabetes. Fasudil significantly increased

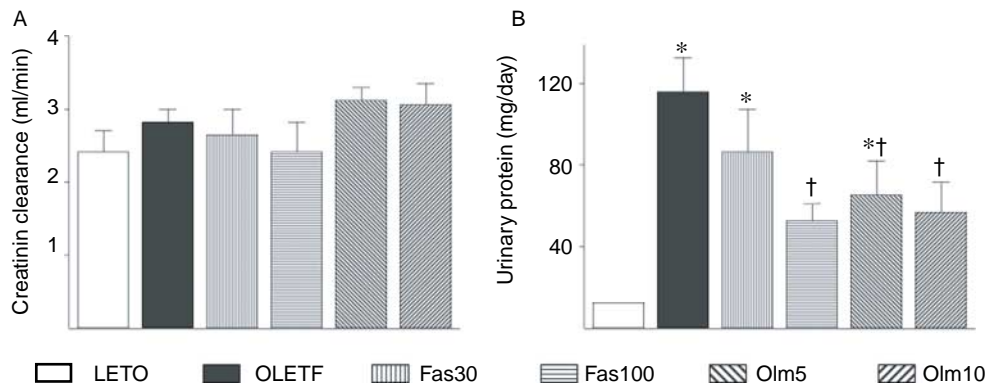


Figure 3 Creatinine clearance (A) and urinary protein (B) in OLETF rats treated with fasudil or olmesartan in Protocol 1. * $P < 0.05$ vs LETO rats, † $P < 0.05$ vs untreated OLETF rats.

serum adiponectin levels in OLETF rats. Peroxisome proliferator-activated receptor (PPAR)- γ regulates adipocyte gene expression and differentiation. Thiazolidinediones, specific ligands for PPAR- γ , also increased the plasma adiponectin levels in insulin-resistant humans and animals,

as well as improving hyperglycemia (Maeda *et al.* 2001, Choi *et al.* 2005). Pioglitazone, a thiazolidinedione, suppressed the Rho/Rho-kinase pathway in aortic tissues from spontaneously hypertensive rats (Wakino *et al.* 2004). Both fasudil and thiazolidinediones might exert a beneficial effect

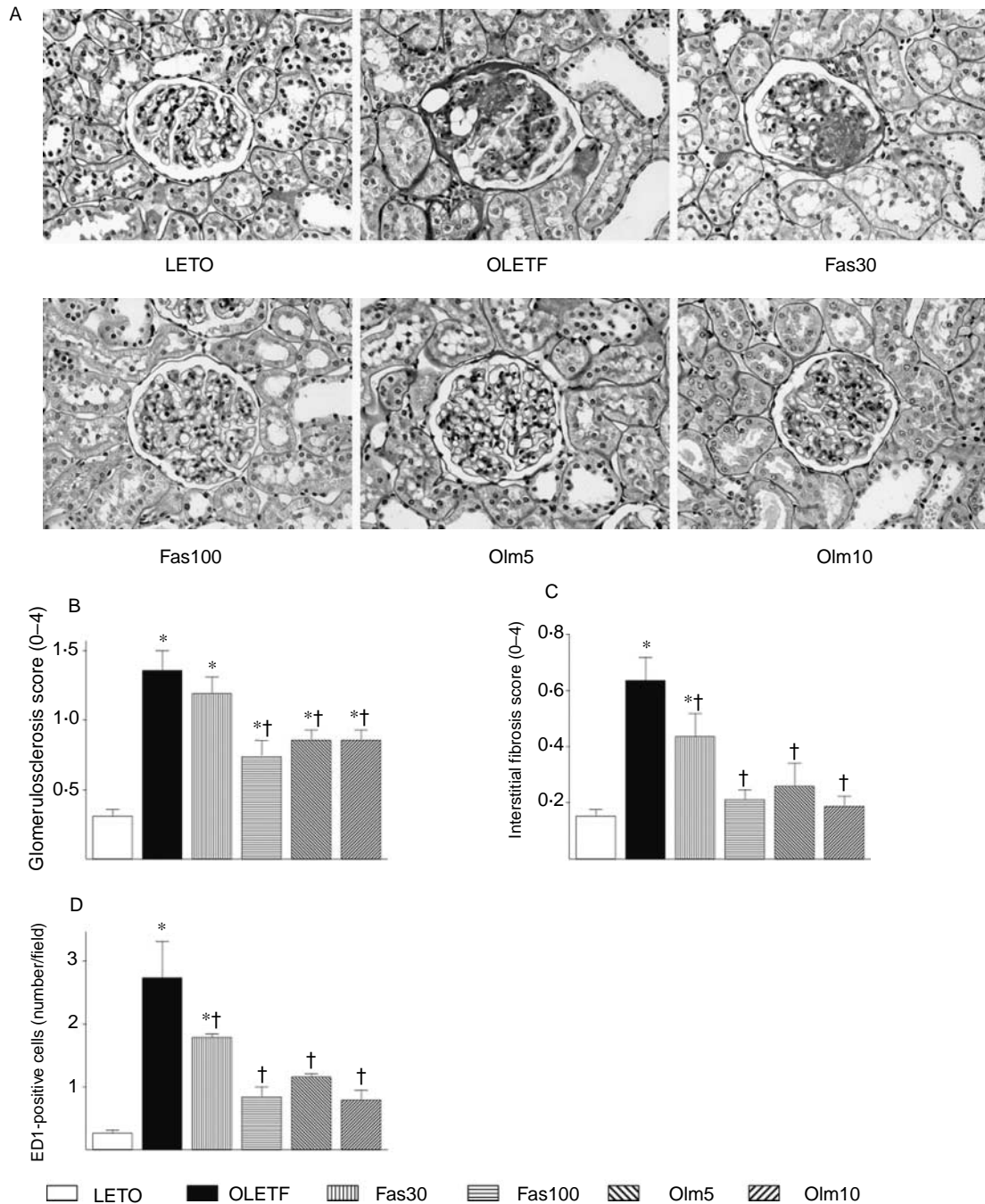


Figure 4 Renal histology and ED1-positive cell infiltration in OLETF rats treated with fasudil or olmesartan in Protocol 1. (A) Glomerular and interstitial changes in PAS-stained sections ($\times 400$). (B and C) Semiquantitative analyses of glomerulosclerosis and interstitial fibrosis in LETO and OLETF rats. (D) ED1-positive cells in the kidneys of LETO and OLETF rats. * $P < 0.05$ vs LETO rats, † $P < 0.05$ vs untreated OLETF rats.

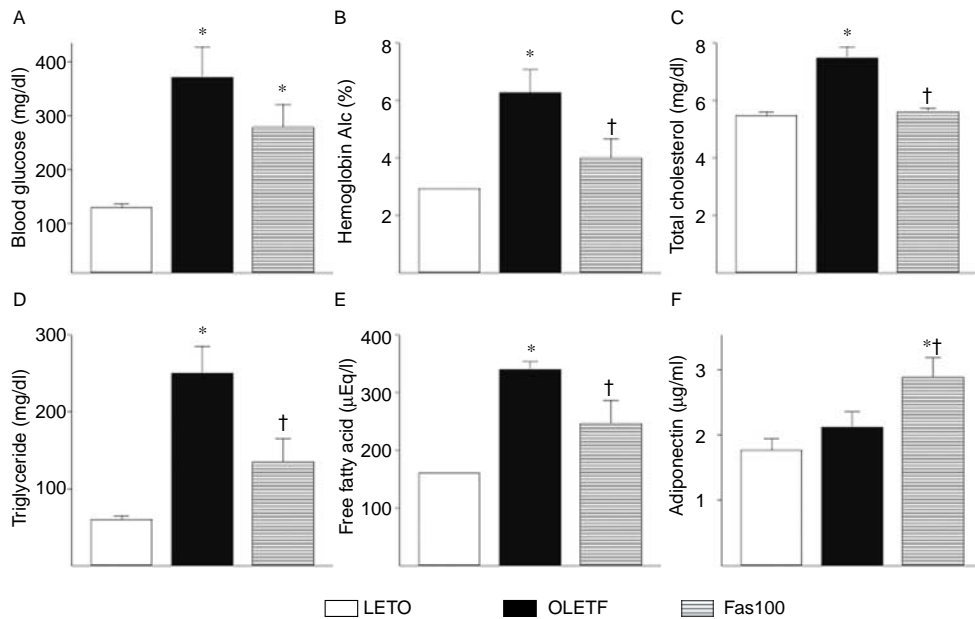


Figure 5 Glucose and hemoglobin A1c (A and B) and lipid metabolism (C–F) in OLETF rats treated with high-dose fasudil for 10 weeks after the development of diabetes in Protocol 2 ($n=5$). Fas100, OLETF rats treated with 100 mg/kg fasudil dissolved in drinking water. * $P<0.05$ vs LETO rats, † $P<0.05$ vs untreated OLETF rats.

on glucose and lipid metabolism, at least in part, through the inhibition of Rho/Rho-kinase activation. Adipocyte hypertrophy induces lipolysis and increases secretion of FFA. The increased levels of FFA might mean an abnormality in adipocyte differentiation. Long-chain fatty acyl-CoA is the activated form of intracellular FFA. Diacylglycerol is generated through esterification of fatty acyl-CoA to glycerol-3-phosphate and activates protein kinase C (PKC). Activated PKC induced insulin resistance by promoting serine phosphorylation of IRS-1 (Hegarty *et al.* 2003). A high plasma concentration of FFA is found in patients with type 2 diabetes. In the present study, serum FFA levels in OLETF rats were significantly higher than those in LETO rats. Administration of high-dose fasudil completely suppressed serum FFA levels in OLETF rats. Kanda *et al.* (2006) reported that fasudil improved glucose metabolism by inhibiting serine

phosphorylation of IRS-1 and the augmentation of insulin signaling in the skeletal muscles of obese Zucker rats. Our results suggest that fasudil might improve metabolic abnormalities not only by improving insulin signaling, but also by correcting adipocyte differentiation.

Olmesartan also improved hemoglobin A1c and serum total cholesterol levels, and increased serum adiponectin levels in OLETF rats. The protective effects of ARB against the development of diabetes have been proven in many clinical trials (Lindholm *et al.* 2002, Julius *et al.* 2004, Yusuf *et al.* 2005). Angiotensin II was reported to activate Rho-kinase in an angiotensin II-induced cardiovascular hypertrophy model (Higashi *et al.* 2003). Therefore, we hypothesized that the effects of ARB on metabolic abnormalities in insulin-resistant models might be due to the inhibition of Rho-kinase activation. However, the activation of Rho-kinase, shown by

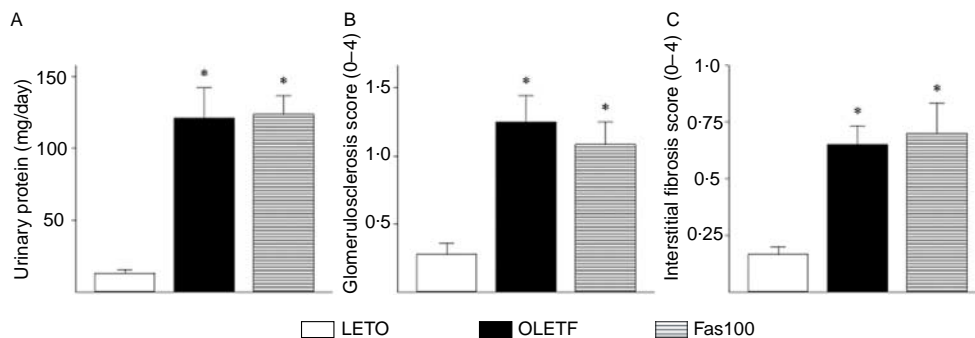


Figure 6 Urinary protein (A) and renal histological changes (B and C) in OLETF rats treated with fasudil (100 mg/kg dissolved in drinking water) for 10 weeks in Protocol 2. * $P<0.05$ vs LETO rats.

phosphorylated MYPT-1 levels, in OLETF rats could not be suppressed by olmesartan. Valsartan, another ARB, improved glucose tolerance through an increase in tyrosine phosphorylation of IRS-1, activation of PI3K, and translocation of GLUT4 to the plasma membrane in skeletal muscle in type 2 diabetic KK-Ay mice (Shiuchi *et al.* 2004). Olmesartan might improve the insulin resistance of OLETF rats by the same mechanisms as valsartan. Olmesartan significantly increased serum adiponectin levels in OLETF rats, but could not affect serum triglycerides or FFA levels. Angiotensin II infusion decreased plasma adiponectin levels in rats and olmesartan ameliorated this hypoadiponectinemia (Ran *et al.* 2006). However, angiotensin II infusion did not affect plasma levels of the other adipocytokines such as leptin or TNF- α . These findings suggest that olmesartan did not improve the adipocyte differentiation itself, although the exact mechanism of increased serum adiponectin by olmesartan remains unknown. Summarizing the above findings, fasudil improved glucose and lipid abnormalities in insulin-resistant models by improving both adipocyte differentiation and insulin signaling in skeletal muscle, more effectively than ARB. The finding that short-term administration of fasudil after the development of diabetes significantly ameliorated the metabolic abnormalities in OLETF rats was notable.

High-dose fasudil and olmesartan inhibited the progression of diabetic nephropathy without affecting blood pressure. In several hypertensive rats, fasudil inhibited the expression of extracellular matrix genes, such as transforming growth factor- β , collagens I and III, oxidative stress, cell proliferation and macrophage infiltration in the kidneys and ameliorated urinary protein, glomerulosclerosis, and tubulointerstitial changes (Kanda *et al.* 2003, Nishikimi *et al.* 2004, Ishikawa *et al.* 2006). Since these changes are also found in diabetic nephropathy, fasudil was expected to have beneficial effects on diabetic nephropathy. In OLETF rats, high-dose fasudil significantly suppressed the urinary protein, glomerulosclerosis, interstitial fibrosis, and macrophage infiltration in the kidneys. However, it was unclear whether these effects were independent of the improvement in metabolic abnormalities. Low-dose fasudil, which could not affect glucose and lipid metabolism, decreased urinary protein by 25%. Glomerulosclerosis score did not change, but the interstitial fibrosis score and macrophage infiltration were significantly inhibited by low-dose fasudil. These results suggest that fasudil may have renoprotective effects in diabetic nephropathy beyond its effects on metabolism in OLETF rats. Our previous study showed that macrophage infiltration in the kidneys was an important event in the development of diabetic nephropathy and was correlated with the pathological changes in OLETF rats (Kikuchi *et al.* 2005). The improvement in interstitial fibrosis might be due to the inhibition of cell migration by fasudil. It was reported that fasudil suppressed renal interstitial fibrosis by inhibiting the macrophage infiltration in rat kidneys with unilateral ureteral obstruction (Satoh *et al.* 2002). In this report, the migration of monocytes *in vitro* was dose dependently

inhibited by fasudil. However, the therapeutic effects of high-dose fasudil initiated in a later stage of diabetic nephropathy were not demonstrated in our study. An additional study using different models may be required to resolve this issue.

In conclusion, long-term treatment with fasudil prevents the development of diabetes and dyslipidemia, at least in part, through the improvement in adipocyte differentiation in insulin-resistant diabetic rats. The use of fasudil from the early phase may prevent diabetic nephropathy. In addition to thiazolidinediones and biguanides, fasudil may become a new therapeutic strategy for insulin resistance, which is characteristic of metabolic syndrome and type 2 diabetes.

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