Effect of TNF-α–Converting Enzyme Inhibitor on Insulin Resistance in Fructose-Fed Rats

Nobuhiko Togashi, Nobuyuki Ura, Katsuhiro Higashiura, Hideyuki Murakami, Kazuaki Shimamoto

Abstract—Insulin resistance is associated with hypertension, obesity, dyslipidemia, and type 2 diabetes. It is well known that tumor necrosis factor (TNF-α) is one of the factors linked to obesity-induced insulin resistance; however, there have been no reports on the role of TNF-α in insulin resistance in nonobese insulin-resistant hypertensives. We tested the hypothesis that TNF-α affects insulin resistance in nonobese insulin-resistant hypertensive fructose-fed rats (FFR) and that a TNF-α–converting enzyme (TACE) inhibitor that blocks TNF-α secretion improves insulin resistance in FFR. Six-week-old male Sprague-Dawley rats were fed either standard chow (control) or fructose-rich chow (FFR) for 6 weeks. For the last two weeks of a six-week period of either diet, the rats were treated with a vehicle (control or FFR) or a TACE inhibitor (100 mg/kg/d of KB-R7785; FFR+TACE-I) in peritoneal injection. At the age of 12 weeks, insulin sensitivity was assessed in all conscious rats by the euglycemic hyperinsulinemic glucose clamp technique. While FFR had higher blood pressure than the control rats (P<0.01), the TACE inhibitor did not change blood pressure. Insulin sensitivity (M-value) was reduced in FFR compared with that in the control rats (16.7±1.1 mg/kg per min and 10.3±0.6 mg/kg per min in the control rats and FFR, respectively, P<0.001), and the TACE inhibitor improved insulin sensitivity to the level of the control rats (14.3±1.2 mg/kg per min in FFR+TACE-I, P<0.01). These data indicate that TNF-α plays a major role in insulin resistance in nonobese insulin-resistant models and also suggest that TACE would be a good target for controlling insulin resistance not only in obese models but also in nonobese insulin-resistant models.

Key Words: tumor necrosis factor ■ insulin resistance ■ fructose ■ hypertension, essential

Insulin resistance is a widespread feature of common atherogenic diseases, including obesity, dyslipidemia, type 2 diabetes, and hypertension, and these diseases are linked to cardiovascular diseases.1 Though the mechanisms of insulin resistance are not yet fully understood, many studies indicate that tumor necrosis factor (TNF)–α plays a major role in the pathogenesis of obesity-induced insulin resistance, resulting from an interaction with the insulin signaling pathway.2 Though TNF-α was discovered as an inflammatory cytokine, it is now known that TNF-α is produced not only in macrophages and lymphocytes but also in adipocytes3 and skeletal muscle cells,4 and that its expression increases in obesity-induced insulin-resistant states. More recent studies using the technique of TNF-α knock-out5 or TNF-α receptor knock-out6 suggest that TNF-α is also an important factor in the regulation of insulin sensitivity.

TNF-α is synthesized as a membrane-anchored 26-kDa precursor, and stimuli triggering inflammation, such as oxidative stress or lipopolysaccharide, cleave it to the secreted 17-kDa form. The cleavage is performed by TNF-α–converting enzyme (TACE),7,8 and much attention is now focused on this enzyme as an ideal target for controlling inflammation. A matrix metalloproteinase inhibitor that blocks TACE activity has recently been found,9 and this inhibitor has been shown to exert an antidiabetic effect by inhibiting tumor necrosis factor-α secretion.10

We previously reported that TNF-α not in adipose tissue but in skeletal muscle tissue is elevated in fructose-fed rats (FFR), and that it probably contributes to insulin resistance in FFR.11 However, we have not obtained a direct evidence that suppression of TNF-α activity improves insulin resistant in FFR. Also, there have been no reports that suppression of TNF-α activity improves insulin resistance in nonobese insulin-resistant hypertensive models. Therefore, we examined the effect of TACE inhibitor on insulin resistance in FFR, a nonobese insulin-resistant hypertensive model.

Methods

General Protocol

Six-week-old male Sprague-Dawley rats (Charles River Japan Inc, Yokohama, Japan) were used in the experiments. All experiments were performed according to the guiding principles of the Physiological Society of Japan. Before any manipulation, all rats were fed standard rat chow containing 60% vegetable starch, 5% fat, and 24% protein (Oriental Yeast Co). They were maintained on a 12-hour light/dark cycle and were given water and chow ad libitum. The rats were acclimated to handling and then randomly divided into three

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groups. The control group (control; n=8) was treated with a vehicle in peritoneal injection once a day for the last 2 weeks of the 6-week standard chow treatment. The FFR group was treated either with 100 mg/kg per day of KB-R7785 (Nippon Organon KK), a TACE inhibitor (FFR+TACE-I; n=8), or with a vehicle (FFR; n=8) in peritoneal injection once a day for the last 2 weeks of the 6-week fructose-rich chow (containing 60% fructose, 5% fat, and 20% protein, No.78463, Teklad) treatment. Systolic blood pressure (SBP) and pulse rate were measured at the end of the treatment period by the tail-cuff method in all groups. At termination, insulin sensitivity was assessed in all conscious rats by the euglycemic hyperinsulinemic glucose clamp technique.

Blood Pressure and Pulse Rate Measurement
SBP and pulse rate were measured in all conscious rats using the indirect tail-cuff method (BP-98A, Softran, Tokyo, Japan) in a 37°C preheated cloth jacket for about 10 minutes. An average of five such recordings was taken as the individual SBP and pulse rate.

Euglycemic Hyperinsulinemic Glucose Clamp Technique
At the end of the treatment period, rats were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg). The right common carotid artery and the right jugular vein were exposed and then cannulated with a polyethylene tube (PE50, Becton Dickinson and Co) for collecting blood samples and administration of the infusate. After overnight fasting (approximately 12 hours), each rat was placed in a foam plastic jacket, which allowed movement of all four limbs and forward vision. At the start of the glucose clamp, fasting blood glucose measurements were performed, and the initial load of insulin (25 mU/kg of humulin R. U-40; Shionogi Pharmaceutical Co) was infused as a bolus, followed by constant infusion of insulin at a rate of 4 mU/kg per min for 147 minutes. During the glucose clamp, 12.5% glucose solution was infused as needed to maintain blood glucose at the fasting level. The samples of 10 μL of arterial blood was obtained at 7-minute intervals for determination of blood glucose. At the end of the glucose clamp, 1.5 mL of blood was withdrawn for measurement of plasma insulin level. The average of the rate of blood glucose infusion for the last 35 minutes was taken as the index of insulin sensitivity (M-value).

Statistical Analysis
All data were analyzed by ANOVA and are expressed as means±SEMs. P<0.05 was considered significant.

Results
Body Weight, Blood Pressure, Pulse Rate and Fasting Blood Glucose
SBP was significantly elevated in FFR compared with that in the control group, and the TACE inhibitor did not significantly change blood pressure (Table 1). There were no significant differences in body weight, pulse rate, or fasting blood glucose among the three groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=8)</th>
<th>FFR (n=8)</th>
<th>FFR+TACE-I (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>385±5</td>
<td>376±6</td>
<td>368±9</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>135±4</td>
<td>155±3*</td>
<td>151±3*</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>338±8</td>
<td>343±8</td>
<td>342±12</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>4.9±0.3</td>
<td>4.6±0.2</td>
<td>5.0±0.2</td>
</tr>
</tbody>
</table>

Values are means±SEM.

*P<0.01 vs control.

Table 1. Characteristics of the Animals at 12 Weeks of Age

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=8)</th>
<th>FFR (n=8)</th>
<th>FFR+TACE-I (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clamp glucose (mM)</td>
<td>5.4±0.1</td>
<td>5.1±0.0</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Clamp insulin (mM)</td>
<td>628±48</td>
<td>605±58</td>
<td>539±27</td>
</tr>
<tr>
<td>M-value (mg/kg/min)</td>
<td>16.7±1.1</td>
<td>10.3±0.6*</td>
<td>14.3±1.2†</td>
</tr>
</tbody>
</table>

Values are means±SEM.

*P<0.001 vs control.

†P<0.01 vs FFR.

Glucose Clamp
Steady-state blood glucose levels and insulin levels during the glucose clamp were similar in all groups (Table 2). The M-value was significantly lower in FFR than in the control rats (16.7±1.1 mg/kg per min and 10.3±0.6 mg/kg per min in the control rats and FFR, respectively, P<0.001). The TACE inhibitor significantly improved the M-value of FFR (14.3±1.2 mg/kg per min in FFR+TACE-I, P<0.01).

Discussion
The results of this study revealed that TNF-α is an important factor in the regulation of insulin resistance in FFR. Although there have been many reports on the role of TNF-α in insulin resistance in obese insulin-resistant models, there have been no reports on the role of TNF-α in insulin resistance in nonobese models.

Since TNF-α was found to be an important factor contributing to obesity-induced insulin resistance, studies have been carried out on the effects of inhibition of TNF-α, and it has been shown that inhibition of TNF-α activity improves insulin resistance. Hotamisligil et al.3 reported that neutralization of TNF-α with 200 μg/rats of recombinant soluble TNF-α receptor immunoglobulin improved insulin resistance in fa/fa (obese) rats. Uysal et al.5,6 also reported that an absence of TNF-α gene or its receptor results in a significant improvement in insulin sensitivity in rodent obese models. It has been reported that blocking TNF-α secretion with a matrix metalloproteinase inhibitor improves insulin resistance in KKAy obese diabetic mice.10

In this study, the TACE inhibitor improved insulin resistance without lowering blood pressure. It has long been reported that insulin resistance and hyperinsulinemia are important factors in essential hypertension,12,13 but our data seem to argue against those notions. However, it has been reported that 8-week treatment with troglitazone (insulin sensitivity enhancer) lowered blood pressure in diabetic hypertensives.14 We reported that 2-week treatment with Tang-Shen-Jiao-Nang, an insulin-sensitizing Chinese medicine that improved insulin resistance in FFR, did not lower blood pressure, but that 4-week treatment lowered it to the level of the control group.15 Therefore, it is thought that 2-week treatment with TACE-I was not sufficient for lowering blood pressure and that longer treatment might lower blood pressure.

It is known that TNF-α is one of the factors affecting obesity-induced insulin resistance and that TNF-α secreted from adipose tissue is elevated in obesity.16 Although it is not
Generally accepted, we believe that TNF-α derived from skeletal muscle is elevated in nonobese insulin-resistant models and that a TACE inhibitor improves insulin resistance through inhibition of TNF-α secretion from the skeletal muscle. Some studies reported that the amount of skeletal muscle TNF-α mRNA is lower than that of adipocyte TNF-α mRNA.4,17 However, we have confirmed that protein levels of TNF-α in skeletal muscle are not so different from those in adipose tissue.11 And even if the amount of TNF-α is small in skeletal muscle, skeletal muscle TNF-α would directly affect glucose metabolism in skeletal muscle tissue, a major tissue for insulin-stimulated glucose uptake. We have reported that TNF-α in skeletal muscle tissue, but not in adipose tissue, is elevated in FFR,11 and it has also been reported that the TNF-α mRNA level in skeletal muscle of insulin-resistant patients was elevated compared with that in the control group, although there was no significant difference between body weights in those groups.4 But we have to keep in mind that the role of skeletal muscle TNF-α on glucose metabolism has not yet been clarified. There have been some in vitro studies that TNF-α increased insulin-stimulated glucose uptake in cultured skeletal muscle cells.18,19 However, there are other reports that TNF-α decreased insulin-stimulated glucose uptake in skeletal muscle cells.20,21 Accordingly there are some discrepancies concerning the role of skeletal muscle TNF-α on insulin-stimulated glucose uptake in vitro. Further in vivo studies on specific inhibition of skeletal muscle TNF-α are needed to prove that skeletal muscle-derived TNF-α, not adipocyte-derived TNF-α, plays a major role in insulin resistance in nonobese insulin-resistant models.

Although the mechanisms of increased TNF-α in skeletal muscle in FFR are not clear, activation of the renin-angiotensin system is thought to be one of the factors upregulating skeletal muscle TNF-α, as we previously reported.11 There have been some reports that angiotensin II upregulates TNF-α in other tissues, including the kidney,22 cardiac fibroblasts,23 and monocytes.24 The regulatory system of TACE activity is also largely unknown, and further investigation is needed to determine whether TNF-α synthesis or TACE activity is upregulated in FFR.

In conclusion, the present results suggest that inhibition of TNF-α activity improves insulin resistance not only in obese models but also in nonobese insulin-resistant models, and that a TACE inhibitor might be a good candidate for clinical insulin-sensitizing medicine.

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References
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