HMG-CoA Reductase Inhibitors Do Not Improve Glucose Intolerance in Spontaneously Diabetic Goto–Kakizaki Rats

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We examined whether 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) improve glucose intolerance in spontaneously diabetic Goto–Kakizaki (GK) rats or not. The fasting blood glucose, plasma insulin, and serum cholesterol levels were significantly higher in GK rats than those in age-matched Wistar rats. All rats were given orally once a day 0.5% carboxymethylcellulose, pravastatin 8 mg/kg, simvastatin 8 mg/kg, or atorvastatin 8 mg/kg. An oral glucose tolerance test (OGTT) was performed before and 3, 6 and 12 weeks after statin treatments. The hyperglycemic response to OGTT in GK rats significantly exceeded that in Wistar rats. The plasma insulin level in GK rats increased with age until 14-week-old (treated for 6 weeks), and then decreased. Glucose intake significantly increased the plasma insulin in almost all rats. The increment of plasma insulin due to OGTT in GK rats appeared to be less than that in Wistar rats, because the basal level was already high in GK rats. Pravastatin, simvastatin, and atorvastatin did not modify changes in blood glucose and plasma insulin induced by glucose intake. In conclusion, long-term treatments of GK rats with statins did not improve glucose intolerance observed during OGTT.

Key words diabetes; Goto–Kakizaki (GK) rat; HMG-CoA reductase inhibitor; oral glucose tolerance test (OGTT); insulin

A number of diabetic patients concomitant with dyslipidemia are now receiving or going to receive statin therapy positively. A post hoc analysis in the West of Scotland Coronary Prevention Study (WOSCOPS) database provided evidence for the protective effect of pravastatin on the development of diabetes.1) Heart protection study of cholesterol-lowering with simvastatin showed highly significant reduction of cardiac event rate in people with diabetes even if they did not have coronary artery disease or high cholesterol concentration.2) Several pleiotropic effects of statins reported may support statin therapy for atherosclerosis observed in diabetes.3,4)

Several clinical studies ascertained a statin therapy to be useful for reduction of atherosclerosis in the patient with diabetes mellitus.5) However, it is not clear whether statins can truly improve diabetes itself in patients, or even in the diabetic model animals. We have demonstrated that pleiotropic effects of statins are not always beneficial on the patient.5,6)

Goto–Kakizaki (GK) rats are produced from normal rats by repetition of selective breeding and established as a model of noninsulin dependent diabetes mellitus.6) The aim of the present study was to examine whether pravastatin, simvastatin and atorvastatin showed to improve glucose intolerance in GK rats assessed by oral glucose tolerance test (OGTT).

MATERIALS AND METHODS

Animals Five-week-old male diabetic GK rats together with age-matched male non-diabetic Wistar rats were purchased from CLEA Japan Inc. (Tokyo, Japan). All animals were housed at 23 ± 1°C under 12-h light and dark cycles, and allowed access to food and water ad libitum. This investigation conforms to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido Pharmaceutical University School of Pharmacy in 2005.

Experimental Protocol GK and Wistar rats were divided into 4 groups, respectively, that is 0.5% carboxymethylcellulose-treated (control), pravastatin-treated, simvastatin-treated and atorvastatin-treated groups. At 8-week-old, treatments of the rats with 0.5% carboxymethylcellulose, 8 mg/kg pravastatin, 8 mg/kg simvastatin, or 8 mg/kg atorvastatin once a day were started. Two days before starting the administration, an OGTT with 5 g/kg glucose intake was performed in overnight fasting rats. The OGTT was repeated 3, 6, and 12 weeks after starting statin administration. In each OGTT experiment, the levels of blood glucose were measured before, and 15, 30, 45, 60, 90, 120, 180 and 300 min after a glucose intake, and the plasma insulin level was determined before and 15 min after a glucose intake. The level of serum cholesterol was determined only before OGTT in 2 d before 8-week-old rats.

Glucose and Insulin Assays Tail-vein blood samples were obtained from the overnight-fasted conscious rats for measurements of blood glucose and plasma insulin levels. The level of glucose in whole blood was determined by the glucose oxidase method of Dahlqvist.7) The level of insulin in plasma was determined as immunoreactive insulin with a Rat insulin [125I]-assay system (Amersham Pharmacia Biotech U.K. Limited, Buckinghamshire, England). HOMA-R was calculated as an indicator of insulin resistance using the following equation; HOMA-R = fasting glucose (mg/dl)×fasting insulin (μU/ml)/405.

Cholesterol Assays Blood was taken from tail-vein. The level of total cholesterol was measured by enzymatic method using a Cholesterol E-test Wako (Wako Pure Chemical Industries).

Statistics All values are expressed as means±S.E. The significances of the differences among groups and within groups were evaluated using repeated measures ANOVA and the paired Student’s t test, respectively, with StatView (SAS Institute Inc., Cary, NC, U.S.A.). A p value of less than 0.05 was considered statistically significant.

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GK rats were significantly higher than those in Wistar rats. The levels of glucose, insulin, and total cholesterol in weight in GK rats were significantly lower than that in Wistar rats. The body weight and the fasting levels of blood glucose, plasma insulin, and serum total cholesterol in Wistar and GK rats 2 d before 8-week-old. The body weight (g) of Wistar and GK rats were measured before and 15 min after a glucose intake (Fig. 1). The OGTT was repeated 3, 6, and 12 weeks after starting statin administration, an OGTT with 5 g/kg glucose intake was performed in overnight fasting day to Wistar (open symbols) and GK rats (solid symbols). Two days before starting the administration, an OGTT with 5 g/kg glucose intake was performed in overnight fasting rats (Before). The OGTT was repeated 3, 6, and 12 weeks after starting statin administration. Data are means±S.E.M. of 5—8 observations. *p<0.05, vs. values in Wistar rats.

### RESULTS

#### Comparison between Wistar and GK Rats before Experiments

Table 1 shows the body weight and the fasting levels of blood glucose, plasma insulin, and serum cholesterol in Wistar and GK rats 2 d before 8-week-old. The body weight in GK rats were significantly lower than that in Wistar rats. The levels of glucose, insulin, and total cholesterol in GK rats were significantly higher than those in Wistar rats.

#### Effects of Stains on Glucose Tolerance

Figure 1 illustrates changes in the blood glucose levels during OGTT in GK and Wistar rats. Hyperglycemic responses in GK rats to OGTT were significantly greater than those in Wistar rats. This value appeared to increase with age until 6 weeks after administration, that is 14-week-old. These increased levels of insulin then tended to decrease at least 12 weeks after statin treatments, that is 20-week-old, although the insulin levels in pravastatin-treated and simvastatin-treated GK rats were still significantly higher than those before starting administration. OGTT for 15-min period significantly increased the plasma levels of insulin in any treated groups in Wistar rats. Increases in plasma insulin in GK rats also observed 15 min after a glucose intake. However, the rate of OGTT-induced insulin secretion in GK rats appeared to be less than that in Wistar rats, because the basal levels of plasma insulin were already high in GK rats. In 20-week-old GK rats, a response of insulin secretion in itself to OGTT was exacerbated further. Pravastatin, simvastatin, and atorvastatin did not modify the fasting levels of plasma insulin in GK rats, but not Wistar rats, gradually but significantly increased with age until 6 weeks after administration, that is 14-week-old. These increased levels of insulin then tended to decrease at least 12 weeks after statin treatments, that is 20-week-old, although the insulin levels in pravastatin-treated and simvastatin-treated GK rats were still significantly higher than those before starting administration. OGTT for 15-min period significantly increased the plasma levels of insulin in any treated groups in Wistar rats. Increases in plasma insulin in GK rats also observed 15 min after a glucose intake. However, the rate of OGTT-induced insulin secretion in GK rats appeared to be less than that in Wistar rats, because the basal levels of plasma insulin were already high in GK rats. In 20-week-old GK rats, a response of insulin secretion in itself to OGTT was exacerbated further. Pravastatin, simvastatin, and atorvastatin did not modify the fasting levels of plasma insulin in any treated groups in Wistar rats. Increases in plasma insulin in GK rats also observed 15 min after a glucose intake. However, the rate of OGTT-induced insulin secretion in GK rats appeared to be less than that in Wistar rats, because the basal levels of plasma insulin were already high in GK rats. In 20-week-old GK rats, a response of insulin secretion in itself to OGTT was exacerbated further.

#### Effects of Stains on Plasma Insulin Response to OGTT

Plasma insulin levels in the control and statin-treated Wistar and GK rats were measured before and 15 min after a glucose intake (Fig. 2). The fasting levels of plasma insulin in GK rats, but not Wistar rats, gradually but significantly increased with age until 6 weeks after administration, that is 14-week-old. These increased levels of insulin then tended to decrease at least 12 weeks after statin treatments, that is 20-week-old, although the insulin levels in pravastatin-treated and simvastatin-treated GK rats were still significantly higher than those before starting administration. OGTT for 15-min period significantly increased the plasma levels of insulin in any treated groups in Wistar rats. Increases in plasma insulin in GK rats also observed 15 min after a glucose intake. However, the rate of OGTT-induced insulin secretion in GK rats appeared to be less than that in Wistar rats, because the basal levels of plasma insulin were already high in GK rats. In 20-week-old GK rats, a response of insulin secretion in itself to OGTT was exacerbated further. Pravastatin, simvastatin, and atorvastatin did not modify the fasting levels of plasma insulin in any treated groups in Wistar rats. Increases in plasma insulin in GK rats also observed 15 min after a glucose intake. However, the rate of OGTT-induced insulin secretion in GK rats appeared to be less than that in Wistar rats, because the basal levels of plasma insulin were already high in GK rats. In 20-week-old GK rats, a response of insulin secretion in itself to OGTT was exacerbated further.

### Table 1. Body Weight, Blood Glucose, Plasma Insulin, and Serum Total Cholesterol in Wistar and GK Rats before Experiments

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>GK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>224±3</td>
<td>194±1*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>75±4</td>
<td>131±6*</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>24±3</td>
<td>49±2*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>77±6</td>
<td>97±3*</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>162±8</td>
<td>113±8*</td>
</tr>
</tbody>
</table>

Data are means±S.E. (n=6). Values were obtained from the rats 2 d before 8-week-old, that is, 2 d before starting carboxymethylcellulose treatments. Because rats were not given any drug yet, the values in the other 3 treated groups were equivalent. *p<0.05, vs. values in Wistar rats.

### Fig. 2. Effects of Stains on Plasma Insulin Level in Wistar and GK Rats

The plasma samples were obtained from the same rats shown in Fig. 1. The plasma insulin level was determined before (solid column) and 15 min after OGTT (solid plus open column). Open columns show the increment of plasma insulin caused by 15 min OGTT. C=control; P=pravastatin; S=simvastatin; and A=atorvastatin. Data are means±S.E.M. of 5—8 observations. *p<0.05, vs. values obtained before OGTT. **p<0.01, vs. the respective values obtained before statin treatments.
Diabetes mellitus is one of the risk factors for atherosclerosis and cardiovascular diseases. It has been demonstrated that a statin therapy can improve atherosclerosis without any exacerbation of diabetes mellitus. A statin therapy is now getting to be strongly recommended for diabetic patients with hypercholesterolemia. A subanalysis of the WOSCOPS showed a significant reduction of incidence of diabetes with pravastatin therapy. In this report, the American Diabetic Association (ADA) definition of diabetes mellitus was used, which requires a fasting blood glucose level of ≥7.0 mmol/l. Pravastatin therapy for 6 months resulted in a 30% reduction in the hazard of becoming diabetic in subjects who had a baseline glucose level of <7.0 mmol/l. Simvastatin and atorvastatin improved insulin resistance assessed by HOMA-R in non-insulin dependent diabetic patients in association with lowering plasma LDL-cholesterol and triglyceride concentrations. Cerivastatin also improves insulin secretion and increases insulin-mediated glucose uptake in the early state of obese type 2 diabetes. These findings lead physicians to impel a statin therapy for patients with diabetes. On the other hand, there are some reports that simvastatin decreases glucose-induced insulin secretion in rat islet $\beta$-cells in vitro, and that atorvastatin worsens glucose tolerance in streptozotocin-induced diabetic rats in vivo.

In the present study, neither improvement nor impairment of the OGTT-induced insulin secretion was observed in GK rats (Fig. 2). The insulin secretion in Wistar rats appeared to get well by a long-term treatment with statins (Fig. 2). Statin treatments did not affect the glucose tolerance appreciably (Fig. 1). In addition, we calculated HOMA-R to assess insulin resistance (Table 2). There was no significant difference in HOMA-R between control and statin-treated groups in Wistar and GK rats. Why did we fail to detect the improvement of insulin resistance by statin treatments? Improvement of insulin resistance by statins is significantly correlated with lowering the plasma triglyceride level. Because the level of triglyceride in GK rats (113±8 mg/dl) is lower than that in Wistar rats (162±8 mg/dl) (our unpublished data), statins may not lower the level of triglyceride in GK rats further. If statins cannot decrease the triglyceride level, these drugs may not affect insulin resistance in GK rats. Another reason is speculated that diabetic state in GK rats may be too severe to detect some difference in OGTT-induced hyperglycemia between the control and statin-treated groups. In the present study, the blood glucose level increased to near 400 mg/dl during OGTT in GK rats.

The beneficial effects of statins have widely been attributed to their effectiveness on reducing LDL cholesterol. However, several studies have indicated direct effects of statins beyond its cholesterol lowering effects. The cholesterol-lowering effect of statins should not be observed in rodents such as mice and rats, because in such animals statin did not induce the low-density lipoprotein receptors in the hepatic cell. This was one of the reasons why we did not measure serum cholesterol level during OGTT. Another reason was that a blood sample obtained was too little to determine all. Our results in the present study may indicate that statins have pleiotropic effects that contribute to the clinical benefits observed.

The dosages of pravastatin, simvastatin and atorvastatin were chosen at 8 mg/kg/d in the present study. The doses of pravastatin and atorvastatin are about 12—24 times higher, and that of simvastatin is about 24—48 times higher than the respective clinical doses. Statins even at the high dosage did not affect glucose tolerance and insulin secretion during OGTT.

In conclusion, diabetes itself in GK rats was not improved by statin treatment, although the treatment did not exacerbate glucose tolerance and insulin secretion. Clinical implication of the present result is that physicians should not be afraid too much but not be expecting too much when they start statin therapy for hypercholesterolemic patients concomitant with diabetes.

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