Nateglinide Suppresses Postprandial Hypertriglyceridemia in Zucker Fatty Rats and Goto–Kakizaki Rats: Comparison with Voglibose and Glibenclamide

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Postprandial hypertriglyceridemia, as well as postprandial hyperglycemia, are important factors contributing to the development of cardiovascular disease in patients with type 2 diabetes. Nateglinide is a recently approved anti-diabetic that suppresses postprandial hyperglycemia by stimulating the early phase of insulin secretion. In the present study, we investigated the effects of nateglinide on postprandial hypertriglyceridemia in obese Zucker fatty (ZF) rats and non-obese diabetic Goto–Kakizaki (GK) rats. Administration of an oral fat load caused marked hypertriglyceridemia with a peak at 2 h in ZF and GK rats. Nateglinide (50 mg/kg) significantly suppressed the increase of plasma triglycerides after fat loading in both types of rat (ΔAUC [0–4 h]: 15±5.3 mg·h/dl for nateglinide vs. 838±100 mg·h/dl for vehicle in ZF rats; p<0.01, 81±22 mg·h/dl for nateglinide vs. 164±17 mg·h/dl for vehicle in GK rats; p<0.01). In contrast, other anti-diabetic agents (voglibose and glibenclamide) did not show a significant effect on the increase of triglycerides after fat loading. The triglyceride components suppressed by nateglinide were mainly at the origin and in the pre β fraction on agarose gel electrophoresis, suggesting that chylomicrons and very low density lipoproteins were decreased. Plasma insulin levels were significantly increased at 30 min in nateglinide-treated rats, but not in voglibose- or glibenclamide-treated rats. These results suggest that nateglinide not only suppresses postprandial hyperglycemia, but also suppresses postprandial hypertriglyceridemia, by promoting rapid and pulsatile insulin secretion in patients with type 2 diabetes.

Key words nateglinide; postprandial; triglyceride; insulin; Zucker fatty rat; Goto–Kakizaki rat

Postprandial hypertriglyceridemia is one of the characteristic pathophysiological abnormalities in type 2 diabetes. Recent epidemiological studies have shown that postprandial triglyceride levels, as well as postprandial glucose levels, are an important contributing factor to development of atherosclerosis and cardiovascular disease. Therefore, the drugs that suppress both postprandial hyperglycemia and postprandial hyperglycemia may be beneficial for preventing the progression of diabetic macrovascular complications.

It is known that α-glucosidase inhibitors reduce postprandial glucose levels by delaying carbohydrate absorption. There should be little effect on lipid metabolism based on this mode of action, though some reports have indicated a slight suppression of postprandial triglyceride levels. A recent clinical study showed that a long-acting sulfonylurea, glibenclamide could improve postprandial hypertriglyceridemia in patients with type 2 diabetes. However, sulfonylureas have an inadequate effect on postprandial hyperglycemia. In addition, it has been pointed out that the optimum insulin secretagogue would be rapidly acting and has a short duration of effect, since the chronic hyperinsulinemia is undesirable.

Nateglinide is a recently approved oral hypoglycemic agent of a new class, which stimulates the early phase of insulin secretion by pancreatic β-cells, and consequently suppresses postprandial hyperglycemia in both animals and patients with type 2 diabetes. In this study, we established postprandial hyperglycemia models using two different diabetic animals, obese Zucker fatty (ZF) rats with insulin resistance and Goto–Kakizaki (GK) rats with impaired insulin secretion. Then we investigated the effects of nateglinide on plasma triglyceride levels after oral loading with fat emulsion, and compared the results with those obtained using an α-glucosidase inhibitor (voglibose) or glibenclamide.

MATERIALS AND METHODS

Animals Male ZF rats and Zucker lean (ZL) rats were purchased from Tokyo Experimental Animals (Tokyo, Japan). GK rats and normal Wistar rats were purchased from Charles River Japan (Yokohama, Japan). This study was reviewed and approved by the Animal Care and Use Committee of Ajinomoto Co., Inc.

Drugs Nateglinide (−)-N-(trans-4-isopropylcyclohexanol carbonyl)-D-phenylalanine; 50 mg/kg, voglibose (0.2 mg/kg), and glibenclamide (1 mg/kg) were suspended in 0.5% methylcellulose and administered to rats via a stomach tube in volume of 10 ml/kg. These doses of the drugs showed a similar suppressive effect on the peak blood glucose levels after oral sucrose or glucose loading of fasted normal rats in our previous study. Control rats were treated with 0.5% methylcellulose alone (the vehicle).

Experimental Design Male Zucker rats and GK rats were used at the age of 12—15 and 32—34 weeks, respectively. After being fasting for 17 h, rats were orally given a fat emulsion (Intralipos, Welfide Co., Osaka, Japan) at a dose of 10 ml (containing 2 g soybean oil)/kg. Drugs were administered orally just before the fat load. In one experiment, rats received drug treatment alone without fat loading. Approximately 200 μl of blood was taken from the tail vein at 0, 30, 60, 120, 180, 240, and 300 min after fat loading, and plasma was separated for biochemical analysis.

Biochemical Analysis Blood glucose levels were deter-
RESULTS

Fasting values were measured after 17 h fasting. Data are expressed as the mean±S.E. *p<0.01 vs. each normal rats.

Table 1. Laboratory Data in Zucker Fatty Rats and GK Rats at the Beginning of the Experiment

<table>
<thead>
<tr>
<th>Rat</th>
<th>Body weight (g)</th>
<th>Fasting plasma triglyceride (mg/dl)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Fasting plasma insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker fatty rats (n=44)</td>
<td>499.6±7.3**</td>
<td>357.0±19.4**</td>
<td>136.8±2.7**</td>
<td>14.8±1.6**</td>
</tr>
<tr>
<td>Zucker lean rats (n=9)</td>
<td>342.7±2.9</td>
<td>55.8±1.4</td>
<td>98.6±2.4</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>GK rats (n=10)</td>
<td>412.9±10.8**</td>
<td>77.1±4.2</td>
<td>197.4±4.8**</td>
<td>1.1±0.1**</td>
</tr>
<tr>
<td>Wistar rats (n=5)</td>
<td>622.7±31.7</td>
<td>113.0±28.1</td>
<td>106.8±3.8</td>
<td>2.4±0.4</td>
</tr>
</tbody>
</table>

Obese ZF Rats

First, we examined the effect of nateglinide in fasted ZF rats. Nateglinide significantly reduced the blood glucose levels by 24% with the nadir at 60 min after treatment in a rapid-acting and short-duration manner, as reported previously (Fig. 1A). Nateglinide also significantly reduced high fasting plasma triglyceride levels by 33%, with the nadir at 60 min after treatment (Fig. 1B). Both effects were transient and these parameters returned to pretreatment levels within 120—180 min.

Effect of Nateglinide on Hypertriglyceridemia after Fat Loading in Obese ZF Rats

Next, we examined the changes of plasma triglyceride levels after oral administration of a fat emulsion to ZF and ZL rats. There was a slight increase of the plasma triglyceride levels after oral fat loading in ZL rats (from 59.0±2.9 mg/dl at baseline to a peak of 92.7±7.4 mg/dl). On the other hand, the plasma triglyceride levels was markedly increased with a peak at 120 min in ZF rats and then returned to the fasting levels at 240 min (Fig. 2).

Nateglinide significantly suppressed the increase of plasma triglycerides in fat-loaded ZF rats at 120 min (p<0.05) and 180 min (p<0.01) (Fig. 3, top). We calculated the area under the curve from baseline (AUC) of plasma triglyceride from 0 min to 240 min. AUC of nateglinide-treated ZF rats was significantly lower when compared with vehicle-treated ZF rats (15±69 mg·h/dl for nateglinide vs. 838±100 mg·h/dl for vehicle; p<0.01). In contrast, the plasma triglyceride levels in voglibose- and glibenclamide-treated ZF rats showed no significant difference compared.
with those in vehicle-treated ZF rats at any time (Fig. 3, middle and lower panels). In voglibose- and glibenclamide-treated rats, $DAUC$ was also not significantly different from that in the vehicle-treated group.

We further analyzed the subfractions of triglycerides after fat loading. Analysis of lipoprotein subfractions by agarose gel electrophoresis showed that the incremental triglycerides at 120 min after fat loading was mainly at the origin and in the pre-$\beta$ subfractions (Fig. 4C), which includes chylomicrons and very low density lipoprotein (VLDL). Nateglinide significantly suppressed the increase both at the origin and in the pre-$\beta$ subfractions (Figs. 4A, B). In contrast, the $\alpha$ and $\beta$ subfractions were almost unchanged from baseline after fat loading and nateglinide did not affect these subfractions.

We measured plasma insulin levels at the same times. Oral fat administration did not change the plasma insulin levels from baseline. In nateglinide-treated rats, a significant, rapid and pulsatile increase of plasma insulin was observed at 30 min, with a return to basal levels at 60 min (Fig. 5). Plasma insulin levels at 30 min were not significantly different in voglibose- and glibenclamide-treated rats compared with vehicle-treated rats (data not shown).

**Effect of Nateglinide on Hypertriglyceridemia after Fat Loading in Non-obese Diabetic GK Rats** Finally, we examined the effect of nateglinide on postprandial lipid metabolism in non-obese GK rats. Oral administration of a fat emulsion induced slight, but significant, hypertriglyceridemia with a peak at 120 min. Nateglinide significantly suppressed the increase of plasma triglyceride levels after fat loading in GK rats (Fig. 6). The $DAUC$ of plasma triglycerides from 0 to 240 min was also significantly reduced in nateglinide-treated GK rats compared with vehicle-treated GK rats ($81\pm22$ mg·h/dl for nateglinide vs. $164\pm17$ mg·h/dl for vehicle; $p<0.01$).
The synthesis of VLDL in the liver. Nateglinide slightly reduced the levels of triglycerides is lipoprotein lipase (LPL), which hydrolyzes the triglyceride component of circulating lipoprotein, and decreases the blood triglyceride levels. Insulin is a major stimulator of the postprandial increase of LPL activity, but other reports have suggested that decreased LPL gene expression is mediated by the increase of insulin in rats with postprandial hyperlipidemia. In some recent transgenic or knockout mice studies have suggested a role of apolipoproteins, receptors for lipoproteins and other enzymes in the mechanisms of lipid abnormalities. Investigation of enzyme activities and/or the expression of these molecules in nateglinide-treated animals is planned to address these issues.

The mechanisms by which nateglinide regulates abnormal postprandial metabolism in these rats are still unclear, but our results suggested the importance of early pulsatile insulin secretion, which is stimulated by nateglinide. The role of early phase insulin has not been clarified since insulin has pleiotropic effects (both directly and indirectly) on exogenous lipoprotein metabolism and endogenous lipoprotein synthesis. One of the well-defined molecules that influence the levels of triglycerides is lipoprotein lipase (LPL), which hydrolyzes the triglyceride component of circulating lipoprotein, and decreases the blood triglyceride levels. Insulin is a major stimulator of the postprandial increase of LPL activity, but other reports have suggested that decreased LPL gene expression is mediated by the increase of insulin in rats with postprandial hyperlipidemia. In some recent transgenic or knockout mice studies have suggested a role of apolipoproteins, receptors for lipoproteins and other enzymes in the mechanisms of lipid abnormalities. Investigation of enzyme activities and/or the expression of these molecules in nateglinide-treated animals is planned to address these issues.

Some recent epidemiological studies have indicated that impaired glucose tolerance is one of the risk factors for cardiovascular disease. Tominaga suggested that macrovascular diseases, unlike microvascular complications, might not be solely due to hyperglycemia. In other words, factors other than hyperglycemia also seem to be related to the development of macrovascular disease. Although further studies will be needed to elucidate the effect of long-term control of postprandial lipid metabolism, our results suggested that restoring early phase of insulin secretion by administration of nateglinide might be a beneficial therapeutic approach for reducing the risk of macrovascular complications in patients with type 2 diabetes.

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REFERENCES