Mechanism of action of hypoglycemic effects of an intestine-specific inhibitor of microsomal triglyceride transfer protein (MTP) in obese rats

Shohei Sakata, Sohei Katsumi, Yasuko Mera, Yukiharu Kuroki, Reiko Nashida, Makoto Kakutani, Takeshi Ohta*

Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan

**ABSTRACT**

Diminished insulin sensitivity in the peripheral tissues and failure of pancreatic beta cells to secrete insulin are known major determinants of type 2 diabetes mellitus. JTT-130, an intestine-specific microsomal transfer protein inhibitor, has been shown to suppress high fat-induced obesity and ameliorate impaired glucose tolerance while enhancing glucagon-like peptide-1 (GLP-1) secretion. We investigated the effects of JTT-130 on glucose metabolism and elucidated the mechanism of action, direct effects on insulin sensitivity and glucose-stimulated insulin secretion in a high fat diet-induced obesity rat model. Male Sprague Dawley rats fed a high-fat diet were treated with a single administration of JTT-130. Glucose tolerance, hyperglycemic clamp and hyperinsulinemic-euglycemic testing were performed to assess effects on insulin sensitivity and glucose-stimulated insulin secretion, respectively. Plasma GLP-1 and tissue triglyceride content were also determined under the same conditions. A single administration of JTT-130 suppressed plasma glucose elevations after oral glucose loading and increased the disposition index while elevating GLP-1. JTT-130 also enhanced glucose-stimulated insulin secretion in hyperglycemic clamp tests, whereas increased insulin sensitivity was observed in hyperinsulinemic-euglycemic clamp tests. Single-dose administration of JTT-130 decreased lipid content in the liver and skeletal muscle. JTT-130 demonstrated acute and direct hypoglycemic effects by enhancing insulin secretion and/or insulin sensitivity.

© 2014 Japanese Pharmacological Society. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**1. Introduction**

Type 2 diabetes mellitus is widely prevalent and has become one of the major health problems worldwide. The disease is a complex metabolic disorder and numerous organs are known to take part in the disease state (1). Therefore, pharmacological therapies that target several organs have been developed to decrease blood glucose level, such as sulfonylureas (targeting pancreatic beta cells), metformin (targeting the liver) and thiazolidinediones (targeting insulin sensitive tissues). glucagon-like peptide-1 (GLP-1)-related therapies (targeting pancreatic beta and alpha cells) and sodium glucose cotransporter 2 inhibitors (targeting kidneys) are recent therapeutic options (2–4). However, impaired insulin secretion and insulin activity are still important determinants for the development of type 2 diabetes.

Microsomal triglyceride transfer protein (MTP) is known to take part in the mobilization and secretion of triglyceride (TG)-rich lipoproteins from enterocytes and hepatocytes (5). JTT-130, a novel intestine-specific inhibitor of MTP suppresses the absorption of dietary fat and cholesterol in the intestine and decreases plasma TG and total cholesterol (TC) without resulting in accumulation of hepatic TG (6,7). JTT-130 suppresses high fat diet-induced obesity and improves glucose and lipid metabolic abnormalities while elevating plasma GLP-1 in Sprague Dawley (SD) rats and Zucker diabetic fatty (ZDF) rats (8–11).

JTT-130 is expected to have another mechanism of action for improving glycemic control other than anti-obesity effects; however, evaluating the direct effects of JTT-130 on glycemic control has been difficult, since repeated administration of JTT-130 decreases
food intake and suppresses obesity. In the present study, we investigated the effect of a single administration of JTT-130 on glucose tolerance, glucose-stimulated insulin secretion and insulin sensitivity by eliminating the anti-obesity effects of the drug in high fat-induced obese SD rats.

2. Materials and methods

2.1. Materials

JTT-130, diethyl-2-({3-dimethylcarbamoyl-4-[[4’-trifluoromethyl ylbiphenyl-2-carbonyl] amino] phenyl]acetoxy)methyl)-2-phen ylmalonate, and pioglitazone were synthesized by Japan Tobacco Inc. (Osaka, Japan). All other reagents used in this study were obtained commercially.

2.2. Animals and diets

Male SD rats (5 weeks of age) were obtained from Charles River Laboratories (Yokohama, Japan), individually housed in an environment with controlled temperature, humidity, and lighting (23 ± 3 °C, 55 ± 15%, and a 12-h light/dark cycle with lights on at 8:00 AM), and were provided a high-fat diet (35%; Oriental Yeast, Osaka, Japan) and water ad libitum after acquisition.

Four separate studies were conducted under the same conditions as described below. SD rats were divided into 2 groups, a control group and a JTT-130 treatment group, according to body weight and food consumption during several days prior to the studies. After subjecting the rats to fasting for 24 h from 10:00 AM, rats were treated with a single administration of vehicle solution (0.5% methylcellulose, 5 mL/kg) or JTT-130 (10 mg/kg) at 10:00 AM, and were fed a high-fat diet for 6 to 8 h. Rats were then fasted overnight and in the next morning oral glucose tolerance tests, hyperglycemic clamp tests or hyperinsulinemic-euglycemic clamp tests were performed in Study 1, 2 and 3. Tissue lipid content was measured under the same conditions in Study 4. All procedures were conducted according to the Japan Tobacco Animal Care Committee’s guidelines.

2.3. Intraperitoneal glucose tolerance test (ipGTT) (Study 1)

ipGTT was performed to the rats (8 weeks of age) after overnight fasting. Glucose solution (1 g/kg) was intraperitoneally administered to rats and blood samples were collected from the tail vein prior to and at 7, 30, 60 and 120 min after glucose loading. Plasma glucose levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7170S, Tokyo, Japan). Plasma insulin level was measured with a rat-insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Plasma GLP-1 levels were evaluated just prior to the ipGTT with a commercial kit (Immuno-Biological Laboratories, Gunma, Japan). The disposition index (DI) was calculated as ∆Ln−30 (change in insulin from 0 to 30 min)/ΔG0−30 (change in glucose from 0 to 30 min) x (1/ fasting glucose x fasting insulin), and was used as an index for insulin secretion.

2.4. Hyperglycemic clamp test (Study 2)

Hyperglycemic clamp tests were performed to the rats (10 weeks of age) after overnight fasting in a separate study. Blood was collected via a carotid artery cannula at −30, −20, −10 and 0 min to measure blood glucose levels using a compact glucose analyzer (Antsense III). After blood sampling at zero minutes, a 25% glucose solution (equivalent to 300 mg/kg) was infused via a jugular vein cannula over one minute using a syringe pump. Blood glucose level was subsequently monitored at two and five minutes after infusion and every five minutes thereafter for up to 90 minutes, and maintained at around 350 mg/dL by adjusting the infusion rate of the 25% glucose solution. Approximately 100 µL of blood was collected in a heparinized tube at −30, −20, −10, 0, 2, 5, and 10 minutes and every 10 minutes thereafter for up to 90 min to measure plasma insulin concentrations. Blood was centrifuged (4 °C, 10,000 × g, five minutes) to obtain plasma. Plasma was stored at −20 °C until measurement. Insulin areas above base line from 0–5 min after glucose infusion were calculated as the first-phase insulin secretion. Moreover, the insulin areas above base line from 10–90 min after glucose infusion were calculated as the second-phase insulin secretion.

2.5. Hyperinsulinemic-euglycemic clamp test (Study 3)

Hyperinsulinemic-euglycemic clamp tests were performed to the rats (7 weeks of age) after overnight fasting in a separate study. A bolus dose of [3-3H] glucose solution (740 kbbq/head) was injected via a jugular vein cannula, followed by continuous infusion at a rate of 7.4 kbbq/min. At 110, 115 and 120 min after the start of the [3-3H] glucose infusion, blood samples (approximately 200 µL) were collected into heparinized tubes via a carotid artery cannula to determine parameters in the basal period. After blood sampling, a bolus dose (0.5 U/kg) of insulin was injected via the jugular vein cannula, followed by continuous infusion of [3-3H] glucose and insulin at the rates of 7.4 kbbq/min and 7 µl/kg/min, respectively. Thereafter, blood samples were collected every five minutes via the carotid artery cannula for blood glucose monitoring using a compact glucose analyzer, and the infusion rate of 25% glucose solution via the jugular vein cannula was adjusted to maintain blood glucose levels at 110 ± 10 mg/dL. Steady-state was defined as the condition in which blood glucose levels were maintained at 110 ± 10 mg/dL without any alteration in glucose infusion rate for 30 min (6 points of continuous glucose monitoring). At the latter 3 points of steady-state, blood samples (approximately 200 µL) were collected into heparinized tubes to determine parameters in the clamp period. Blood samples were immediately centrifuged at 10,000 × g for five minutes at 4 °C to obtain plasma. Plasma was stored at −20 °C until the measurement of plasma parameters. The rate of glucose disappearance (Rd) and hepatic glucose production (HGP) were calculated using Steele’s equation from tracer data as previously described (12). In the basal state, HGP was calculated as Rd. HGP during the clamp study was calculated as the difference between Rd and the infusion rate of exogenous glucose.

2.6. Measurement of tissue lipid content (Study 4)

Lipid content in tissues, such as the liver and skeletal muscle of the rats (7 weeks of age), was measured after overnight fasting in a separate study. After adding 1.5 mL of methanol, liver slices were sufficiently homogenized using one zirconia ball (Y2Z® Ball, Nikkato Corp.) and a mixer mill (MM300, Retsch Co., Ltd.). Four hundred µL of chloroform was added to 400 µL of homogenates and mixed. Mixtures were centrifuged at 10,000 × g for 5 min and supernatants were collected. Supernatants were evaporated under a nitrogen gas flow and residues were dissolved in 500 µL of 2-propanol. TG and free fatty acid (FFA) concentrations in the 2-propanol solution were measured by an enzymatic colorimetric method using an automatic biochemical analyzer or microplate reader and hepatic TG and FFA content were calculated.

After adding 1 mL of methanol, skeletal muscle slices were sufficiently homogenized using one zirconia ball (Y2Z® Ball, Nikkato Corp.) and a mixer mill (MM300, Retsch Co., Ltd.). Eight hundred µL of chloroform was added to 800 µL of homogenates and...
mixed. Mixtures were centrifuged at 10,000 \times g for 5 min and supernatants were collected. Supernatants were evaporated under a nitrogen gas flow and residues were dissolved in 100 \mu L of 2-propanol. TG concentrations in the 2-propanol solutions were measured by an enzymatic colorimetric method using an automatic biochemical analyzer or microplate reader and TG content in skeletal muscle was calculated.

2.7. Statistical analysis

Data were expressed as mean values ± s.d or + s.d. Student’s t-tests or Aspin-Welch tests were used to determine statistical significance. P < 0.05 was considered statistically significant. In hyperglycemic clamp test (study 2), statistical analysis was performed in the first-phase insulin secretion and the second-phase insulin secretion. In hyperinsulinemic-euglycemic clamp test (study 3), the analysis was performed in each value of steady-state.

3. Results

3.1. Intraperitoneal glucose tolerance test

As previously reported (9), a single administration of JTT-130 increased plasma GLP-1 levels in this study (Fig. 1). There were no significant effects on body weights prior to testing (Control; 298.0 ± 18.9 g, JTT-130; 292.2 ± 8.6 g, p = 0.51) and changes in body weights (Control; −4.9 ± 4.3 g, JTT-130; −8.0 ± 4.3 g, p = 0.24) during the experiment, thus an evaluation of the effects of JTT-130 on glucose metabolism without influencing anti-obesity effects was considered possible. Changes in plasma glucose and insulin levels in ipGTT are shown in Fig. 2. In ipGTT, plasma glucose levels were significantly lower in the JTT-130 treatment group than in the control group (Fig. 2(A)), showing that JTT-130 improved glucose tolerance. Plasma insulin levels in the JTT-130 treatment group did not significantly increase compared with the control group; however, DI tended to increase (Control; 3.05 ± 0.40 (\times 10^{-5} (mg/dL)^{-2}), JTT-130; 7.74 ± 2.25 (\times 10^{-5} (mg/dL)^{-2}, p = 0.17).

3.2. Hyperglycemic clamp test

Since hyperglycemia induced by glucose loading in ipGTT was transient, detecting the effects of JTT-130 on insulin secretion may be insufficient. Thus, we examined the effects of JTT-130 on glucose-stimulated insulin secretion under sustained hyperglycemic conditions using hyperglycemic clamp techniques in the next study. There were no significant effects on body weights prior to testing (Control; 428.2 ± 47.3 g, JTT-130; 431.5 ± 20.0 g, p = 0.87), and changes in body weights (Control; −2.3 ± 5.5 g, JTT-130; −1.8 ± 2.9 g, p = 0.85) and food consumption (Control; 14.6 ± 1.3 g, JTT-130; 13.5 ± 2.2 g, p = 0.28) during the experiment. Prior to the initiation of glucose infusion, there were no significant changes in plasma insulin levels between both groups, suggesting that JTT-130 did not affect insulin secretion in the fasting state. After the initiation of glucose infusion, plasma glucose levels were maintained at approximately 350 mg/dL in both groups from 50 min after glucose infusion until the end of the study. An increase in plasma insulin levels corresponding to the increase in blood glucose was observed in both groups and was almost similar between the two groups at two minutes after the start of glucose infusion (first-phase insulin secretion). In contrast, the mean insulin level from 10 to 90 min (second-phase insulin secretion) tended to increase in the JTT-130 group compared with the control group (p = 0.068), showing that a single administration of JTT-130 tended to increase glucose-stimulated insulin secretion (Fig. 3).
3.3. Hyperinsulinemic-euglycemic clamp test

To elucidate the mechanism of action of JTT-130 responsible for ameliorating impaired glucose tolerance without affecting plasma insulin levels, a hyperinsulinemic-euglycemic clamp test to assess the effects of JTT-130 on insulin sensitivity was performed. There were no significant effects on body weights prior to testing (Control: 274.9 ± 14.2 g, JTT-130: 277.0 ± 13.7 g, p = 0.80), and changes in body weights (Control: −1.2 ± 4.3 g, JTT-130: 1.4 ± 3.8 g, p = 0.33) and food consumption (Control: 13.8 ± 3.0 g, JTT-130: 11.7 ± 1.8 g, p = 0.20) during the experiment. Compared with the basal period, plasma insulin levels were maintained at higher levels during the clamp period and there were no significant differences between both groups (Table 1). The plasma glucose levels in both groups were maintained at 110 ± 10 mg/dL at 140 min and after (Fig. 4A). Glucose infusion rates in the JTT-130 group were significantly higher than the control group (Table 1 and Fig. 4B). A tracer study showed that Rd was significantly higher and HGP was significantly lower in the JTT-130 treatment group compared with the control group, indicating that a single administration of JTT-130 improved insulin sensitivity, both in the liver and peripheral tissues (Table 1).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>JTT-130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>Basal</td>
<td>135 ± 4</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>Basal</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>12.7 ± 1.6</td>
</tr>
<tr>
<td>GIR (mg/kg/min)</td>
<td>Basal</td>
<td>5.4 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td>Rd (mg/kg/min)</td>
<td>Basal</td>
<td>11.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>6.4 ± 2.3</td>
</tr>
<tr>
<td>HGP (mg/kg/min)</td>
<td>Basal</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>6.4 ± 2.3</td>
</tr>
</tbody>
</table>

Data represent mean values ± s.d. (n = 6). *p < 0.05, **p < 0.01; significantly different from control group. Each parameter was measured or calculated during the last 15 min of the clamp.

Fig. 3. Changes in blood glucose (A) and plasma insulin (B) levels in the control group and JTT-130 treatment group in hyperglycemic clamp tests. Data represent mean values ± s.d. (n = 7). Insulin areas above base line from 0–5 min are considered as first-phase insulin secretion, and the areas above base line from 10–90 min are considered as second-phase insulin secretion.

Fig. 4. Changes in blood glucose levels (A) and glucose infusion rate (B) in the control group and JTT-130 treatment group in hyperinsulinemic-euglycemic clamp tests. Data represent mean values ± s.d. (n = 5–6).
3.4. Effects on tissue lipid content

Since JTT-130, an intestine-specific MTP inhibitor, was considered to inhibit lipid absorption in the small intestine, JTT-130 was expected to affect tissue lipid content that has been reported to be associated with insulin sensitivity in tissues. Thus, the effects of JTT-130 on lipid content (TG and FFA) in the liver and skeletal muscle were assessed. There were no significant effects on body weights prior to testing (Control: 288.0 ± 16.6 g, JTT-130: 286.5 ± 18.4 g, \( p = 0.86 \)), and changes in body weights (Control: −6.7 ± 3.6 g, JTT-130: −6.9 ± 6.5 g, \( p = 0.95 \)) and food consumption (Control: 14.8 ± 2.0 g, JTT-130: 13.4 ± 3.1 g, \( p = 0.30 \)) during the experiment. TG and FFA content in the liver and TG content in the skeletal muscle were lower in the JTT-130 treatment group than in the control group. In particular, the effects on FFA content in the liver and TG content in the soleus muscle were significant (Fig. 5).

4. Discussion

To date, several oral diabetic agents, such as pioglitazone, which enhances insulin sensitivity of insulin target organs, and GLP-1 analogs or dipeptidyl peptidase-4 inhibitors, which enhance glucose-stimulated insulin secretion from the pancreas, have been developed for type 2 diabetes management (13–15). However, to our knowledge, there are no reported agents that directly affect insulin sensitivity and glucose-stimulated insulin secretion.

In the present study, we intended to evaluate the effects of a single administration of JTT-130 on glucose metabolism by eliminating its anti-obesity effects. As shown in the previous report, the significant influence was observed on food intake for 24 h after a single administration of JTT-130 (8). However, a single administration of JTT-130 (and subsequent food intake for 6 to 8 h) had no significant effects on food intake and body weight, indicating the ability to assess the effects of JTT-130 on glucose metabolism in the present series of studies. In ipGTT after fasting overnight, JTT-130 improved glucose tolerance, and increased DI while elevating GLP-1 levels. DI quantifies the nature of the interactions between pancreatic insulin secretion and insulin sensitivity, and the index is considered to be predictive of development of type 2 diabetes (16,17). Indeed, JTT-130 tended to enhance glucose-stimulated insulin secretion under sustained hyperglycemic conditions; however, another mechanism of action of JTT-130 for ameliorating glucose tolerance was considered. JTT-130 was found to improve insulin sensitivity in the liver and peripheral tissues in hyperinsulinemic-euglycemic tests. Thus, one possible reason as to why the effects on insulin secretion were not significantly detected in ipGTT was the dual effect of JTT-130 on enhanced glucose-stimulated insulin secretion (elevated insulin secretion) and enhanced insulin sensitivity (suppressed insulin secretion).

Increased tissue lipid content in the liver and skeletal muscle (ectopic fat deposition) has been widely reported as reducing insulin sensitivity and being associated with insulin resistance in animal models and humans (18–21). We found that JTT-130 decreased lipid content in the liver and skeletal muscle, suggesting that these effects may be the possible mechanism for the effects of JTT-130 on insulin sensitivity. There are some reports in which the insulin resistance can be induced by relatively short-time infusion of lipids in rodents and human (22,23), and it is
considered that JTT-130 improved insulin resistance in tissue via decreased exposure of fat to the tissues by blockade of fat absorption.

It is noteworthy that these effects of JTT-130 (enhanced glucose-stimulated insulin secretion and insulin sensitivity) can be attributed to one mechanism of action of JTT-130, the blockade of fat absorption in the small intestine. Blockade of fat absorption by JTT-130 causes increases in fatty acid content in the lumen of the small intestine and leads to the elevation of the plasma GLP-1 levels. Suppressed fat absorption in the intestine may limit the supply of fat in circulation and subsequently in tissues, such as the liver and skeletal muscle.

In summary, these findings demonstrate that a single administration of JTT-130, an intestine-specific MTP inhibitor, enhanced glucose-stimulated insulin secretion and insulin sensitivity in high fat-induced obese SD rats. These results suggest that pharmacological inhibition of fat absorption in the small intestine may be a useful therapeutic target for the treatment of type 2 diabetes mellitus.

Conflicts of interest

All the authors indicated no potential conflicts of interest.

References