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Antidiabetic effect of chronic administration of JTT-608, a new hypoglycemic agent, in diabetic Goto–Kakizaki rats

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Abstract

We investigated the chronic effect of a new antidiabetic agent, *trans*-4-(methylcyclohexyl)-4-oxobutyric acid (JTT-608), in Goto–Kakizaki rats, a genetic model of non-obese type II diabetes mellitus. The rats were fed a liquid meal, three times a day, for 12 weeks. The rats were treated orally with JTT-608 (10–100 mg/kg) 10 min before each meal. Fasting blood glucose, triglyceride and hemoglobin A1c levels were reduced by JTT-608 at all dose levels during the experimental period. Blood glucagon-like peptide-1 level with 100 mg/kg JTT-608 increased at the end of the treatment period. JTT-608 (30–100 mg/kg) reduced urinary protein levels after administration for 5–12 weeks. In Goto–Kakizaki rats showing slight diabetic renal lesions, pathological examination revealed that JTT-608 reduced the incidence of vacuolation in renal tubules. JTT-608 (30–100 mg/kg) ameliorated the reduced motor nerve conduction velocities observed in the Goto–Kakizaki rats after administration for 12 weeks. We conclude that chronic administration of JTT-608 produces good blood glucose control and gradually arrests the development of diabetic neuropathy and nephropathy.

Keywords: JTT-608; Antidiabetic agent; Hemoglobin A1c; Goto–Kakizaki rat; Neuropathy; Nephropathy

1. Introduction

The results of the United Kingdom Prospective Diabetes Study were reported in 1998. The study indicated that lowering blood glucose levels in patients with type 2 diabetes reduces the risk of clinical complications, especially in diabetic microangiopathy (Turner, 1998). The clinical benefit of intensive control of blood glucose levels was clearly established. Data from the United Kingdom Prospective Diabetes Study demonstrated that improving glycemic control reduces the risk of microvascular complications from type 2 diabetes, such as diabetic retinopathy, nephropathy and peripheral neuropathy (Turner et al., 1999). Blood glucose concentration peaks during the post-prandial period, placing type 2 diabetic patients at high risk of cellular and tissue damage, as shown by abundant experimental data obtained from tissue cultures and as evidenced by chronic hyperglycemia-induced complications in the type 2 diabetic patients (Home, 1999; DeFronzo, 1999). Improved prandial glucose metabolism leads to an overall improvement in glycemic control (Landgraf, 1999; Lehmann, 2001).

Sulfonylurea derivatives are one of the widely used oral hypoglycemic agents for the clinical management of patients with type 2 diabetes (Leibowitz and Cerasl, 1996; Groop, 1992; Gerich, 1989). However, sulfonylurea derivatives can cause severe and prolonged hypoglycemia (Ferner and Neil, 1988; Aspuland et al., 1983) because of their long duration of action and glucose-independent action. Furthermore, it was reported that long-term treatment with sulfonylurea derivatives resulted in pancreas degeneration (Kawai et al., 1991; Dunber and Foa, 1974; Sodoyers et al., 1970) and caused secondary failure in diabetes mellitus patients (Antonio et al., 1994; Harrower, 1994; William et al., 1972). To avoid these effects from sulfonylurea derivatives and achieve good control of blood glucose in patients with type 2 diabetes, we developed an innovative drug, *trans*-4-(methylcyclohexyl)-4-oxobutyric acid (JTT-608), which repairs the defect of glucose-stimulated insulin secretion, especially the first phase of insulin secretion in the pancreas, and improves post-prandial hyperglycemia. We have already found that JTT-608 enhanced insulin secretion in mouse insulinoma (MIN) six cells in a glucose concentration-dependent manner (Furukawa et al., 1999). Also, in isolated, perfused pancreas or pancreatic islets of rats, JTT-608 enhanced insulin secretion at high glucose concentrations,

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but not at low concentrations, and augmented the secretion by inducing and increasing Ca^{2+} efficacy (Ohta et al., 1999b; Mukai et al., 2000). Additionally, it is thought that JTT-608 might have mechanisms different from those of sulfonylurea derivatives, as JTT-608 dose not bind to sulfonylurea receptors (Furukawa et al., 1999). Also, a single oral dose of JTT-608 improved glucose tolerance with enhancement of insulin secretion in type 2 diabetic rats such as neonatal streptozotocin-induced diabetes mellitus rats (Ohta et al., 1999a) and Goto–Kakizaki rats (Ohta et al., 1999b).

Estimation of the chronic effect of a drug in type 2 diabetic animal models is an important theme for the development of an antidiabetic agent. Goto–Kakizaki rats, a genetic model of non-obese type 2 diabetes mellitus, show durable hyperglycemia and neuropathy from an early stage (Wada et al., 1999; Yagihashi et al., 1982). These animals exhibit slight nephropathy, which does not develop fully (Vesely et al., 1999; Yagihashi et al., 1978), while retinopathy is not observed during their lifetime (Agardh et al., 1997; Sone et al., 1997). In the present study, we investigated the chronic antidiabetic effect of JTT-608, concentrating on long-term blood glucose control, in Goto–Kakizaki rats.

2. Materials and methods

2.1. Chemical

JTT-608 was synthesized at Japan Tobacco, Central Pharmaceutical Research Institute (Osaka, Japan).

2.2. Animals, feeding and drug administration

This experiment complied with the Guidelines for Animal Experimentation of our laboratories. Goto–Kakizaki rats were obtained from Charles River Japan (Yokohama, Japan). Age-matched Wistar rats were used as normal control animals. The animals were fed a liquid meal (composition: 0.273% L-lysine, 0.164% methionine, 3.861% corn oil, 14.570% olive oil, 2.732% vitamin mixture, 5.464% mineral mixture, 1.475% ethyl linoleate, 38.600% sucrose, 32.845% lactalbumin, 0.016% DL-α-tocopherol acetate; total energy 4574.27 kcal/kg; Nihon Nosan, Yokohama, Japan) in a light/dark reversed room from 5 weeks of age. At 7 weeks, the animals were divided into groups by checking the fasting blood glucose and hemoglobin A1c levels. Forty Goto–Kakizaki rats were prepared for this experiment and 32 of them were randomized into groups. After assignment, Goto–Kakizaki rats were treated orally with JTT-608 (10–100 mg/kg), three times a day (administration time: 9:00, 13:00, 17:00), for 12 weeks. Ten minutes after every administration of JTT-608, liquid meal was given orally. Liquid meal was prepared to be about 4 kcal/ml using distilled water. Treatment volume was 5 ml in Goto–Kakizaki rats and 6–8 ml in Wistar rats. Aside from the liquid meal-fed rats, the *ad libitum* fed controls (Charles River Formula-1, Oriental Yeast, Tokyo, Japan) were prepared and mean food consumption per day calculated every week. Liquid meal of equal energy level to the mean food consumption was given orally, divided into three meals a day. Woodchips were placed in each cage to avoid overgrowth of the front teeth of the rats.

2.3. Blood sample collection and biochemical analyses

During the treatment period, fasting blood glucose, hemoglobin A1c, insulin and triglyceride levels were measured at 4-week intervals in blood samples taken immediately before the first administration on that day. Goto–Kakizaki rats were fasted for 16 h before blood sample collection. Glucose and triglyceride concentrations were determined by the hexokinase method, using commercial kits (Boehringer Mannheim, Tokyo, Japan). Hemoglobin A1c levels were determined by an affinity column method, using a commercial kit (Seikagaku, Tokyo, Japan). Insulin concentrations were determined by a two-antibody procedure using a radioimmunoassay kit (Shionogi, Osaka, Japan). After 12-week administration, we performed a meal tolerance test in order to confirm an improvement of post-prandial glucose tolerance by JTT-608. The rats were fasted for 20 h before the meal tolerance test. JTT-608 was administered orally 10 min before liquid meal loading. As on the previous day, a draft quantity of the liquid meal administration was loaded. The treatment volume was 5 ml (4 kcal/ml). Blood samples were taken before meal loading, 0.5 and 2 h after meal loading, and blood glucose concentrations were measured. We calculated glucose areas above baseline from 0 to 2 h. We also collected blood samples at the time of anatomic inspection and glucagon-like peptide-1 concentrations were determined by the bead solid phase method using a radioimmunoassay kit (Amersham, Tokyo, Japan).

2.4. Determination of urinary protein

After 5, 9 and 12 weeks of administration, the Goto–Kakizaki rats were housed in metabolic cages for 16 h and their urine was collected. Protein concentrations in urine were determined using a commercial kit (Otsuka, Tokushima, Japan). Creatinine concentrations were determined by colorimetry using a commercial kit (Kimura Works, Osaka, Japan). The urinary protein content was calculated in terms of creatinine concentration (protein/urinary creatinine).

2.5. Determination of nerve conduction velocity

After 12-week administration, motor nerve conduction velocities in rats were determined according to previously described methods (Ido et al., 1994; Miyoshi and Goto, 1973; Spüler et al., 1987) with some modifications. JTT-608 and liquid meals were not given on the day of the test. The animal was lightly anaesthetized with diethyl ether, restrained using a restraint vessel, and the tail was placed on a heated pad maintaining a surface temperature of 37 °C.

The tail nerve was stimulated by a stimulator (SEN-3201, intensity = 100 V, Nihon-Koden, Tokyo, Japan) through a bipolar electrode (M·T Giken, Tokyo, Japan) placed 1 cm from the anus. It was used as a bipolar electrode at two points of recording: the first 4 cm from the stimulus electrode and the second 4 cm from the first recording electrode. The muscle action potentials were amplified (AVB-11, Nihon-Koden) and displayed with an amplifier-oscilloscope (VC-10, Nihon-Koden). Groups of 32 potentials were averaged using an averager (DAT-1100, Nihon-Koden) and recorded on a chart recorder (RTA-1200, Nihon-Koden). Conduction velocity was calculated from the delta latency between two recording electrodes divided by the distance of 4 cm.

2.6. Pathological analyses

The rats were killed after the 12-week administration period. The following tissues were collected for histopathological examination: kidneys, pancreas, eyes and aorta. Tissues were taken from all animals and preserved in 10% neutral buffered formalin (eyes were preserved in 2.5% glutaraldehyde solution), embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopic evaluation. Additional sections of kidney were stained with periodic acid Schiff's reagent. Immunohistochemistry was performed on sections of pancreas with an anti-insulin antibody (Dako, Japan), by indirect staining using peroxidase-conjugated anti-guinea pig immunoglobulins (Dako). The remainder of each pancreas was used for determination of insulin content.

2.7. Statistical analysis

All results except those from pathological analyses were expressed as the means ± S.E.M. Statistical analyses of differences between mean values were performed using Dunnett's two-tailed test or Student's *t*-test. Pathological analyses were evaluated with the non-parametric Dunnett's test. Differences were defined as significant at $P < 0.05$.

3. Results

3.1. Effect of JTT-608 on glycemic control and other biochemical parameters in Goto–Kakizaki rats

During the experimental period, fasting blood glucose and hemoglobin A1c concentrations in Goto–Kakizaki rats

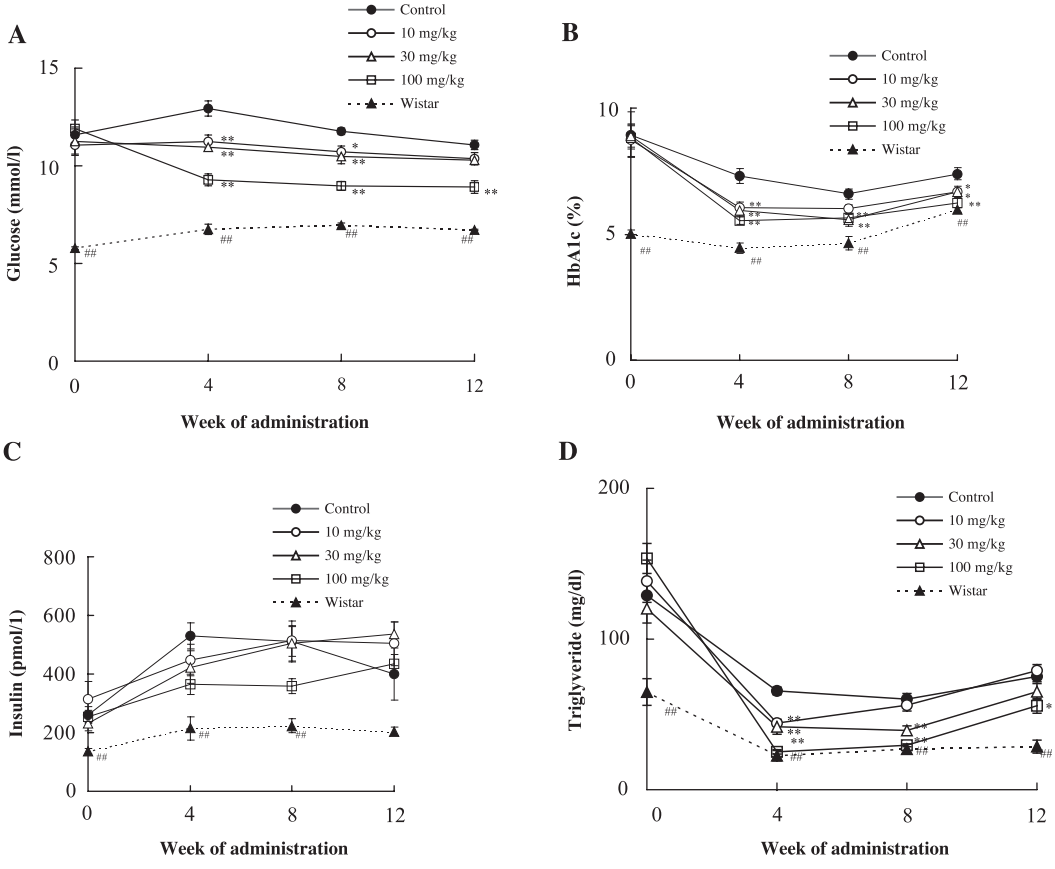

Fig. 1. Effects on blood glucose (A), hemoglobin A1c (B), insulin (C) and triglyceride (D) levels in Goto–Kakizaki rats with chronic administration of JTT-608. Data are means ± S.E.M. (Goto–Kakizaki rats; $n = 7–8$, Wistar rats; $n = 5$). *$P < 0.05$, **$P < 0.01$; significantly different from the control, Dunnett's two-tailed test. $^{\#\#}P < 0.01$; significantly different from the control, Student's *t*-test.

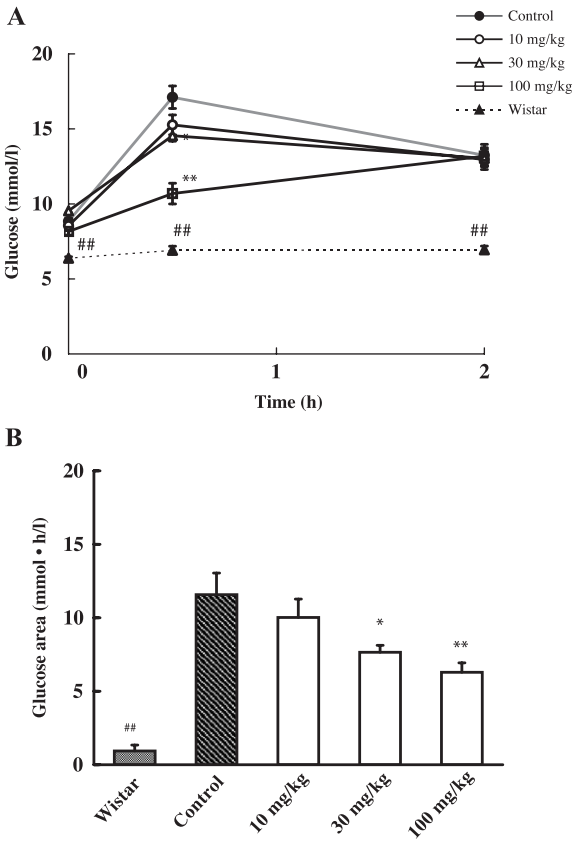

Fig. 2. Effect of JTT-608 on blood glucose levels (A) and glucose areas (B) in meal-loaded Goto–Kakizaki rats. Glucose areas were calculated above baseline from 0 to 2 h on (A). Data are means ± S.E.M. (Goto–Kakizaki rats; $n=7–8$, Wistar rats; $n=5$). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control, Dunnett's two-tailed test. $^{\#\#}P<0.01$; significantly different from the control, Student's *t*-test.

were high, compared to those in Wistar rats. Fasting blood glucose levels were reduced with doses 10 mg/kg or higher of JTT-608, compared to levels in the control group (Fig. 1A). Glucose levels decreased from 4 to 8 weeks after

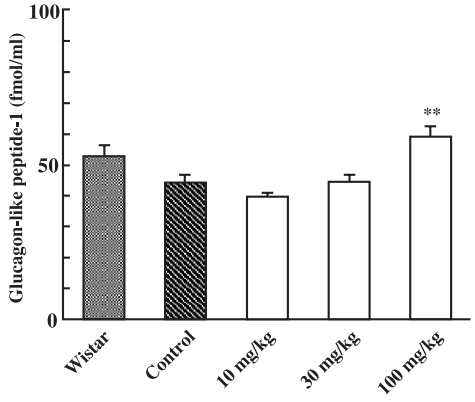

Fig. 3. Effect on glucagon like peptide-1 levels in Goto–Kakizaki rats after 12 weeks of JTT-608 administration. Data are means ± S.E.M. (Goto–Kakizaki rats; $n=5–8$, Wistar rats; $n=5$). $^{**}P<0.01$; significantly different from the control, Dunnett's two-tailed test.

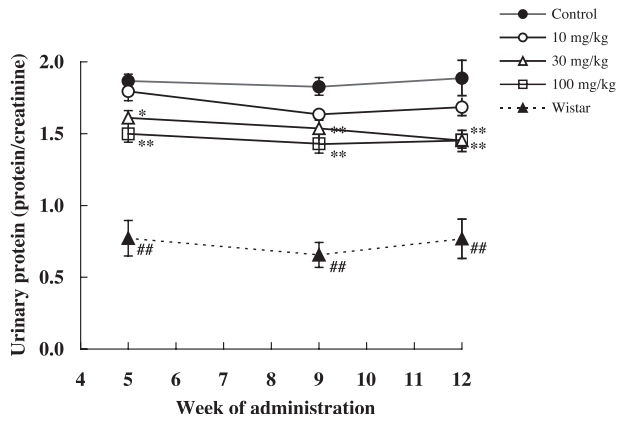

Fig. 4. Effect on urinary protein in Goto–Kakizaki rats after 5, 9 and 12 weeks of JTT-608 administration. Data are means ± S.E.M. (Goto–Kakizaki rats; $n=6–8$, Wistar rats; $n=5$). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control, Dunnett's two-tailed test. $^{\#\#}P<0.01$; significantly different from the control, Student's *t*-test.

administration of 10 or 30 mg/kg JTT-608, and the glucose levels were reduced during all treatment periods in animals given 100 mg/kg JTT-608. Hemoglobin A1c levels before administration of JTT-608 were about 9% in controls and the levels decreased to about 7.5% after administration for 4 weeks. This decrease persisted at 12 weeks after the start of administration. Hemoglobin A1c levels were reduced at all doses of JTT-608, compared to those in the control group (Fig. 1B). The hemoglobin A1c levels in the 10 mg/kg JTT-608 group decreased from 4 weeks after administration and the levels in the 30 and 100 mg/kg JTT-608 groups were reduced during all treatment periods, from 4 to 12 weeks. JTT-608 produced good blood glucose control during the experimental period.

Fasting insulin and triglyceride levels in Goto–Kakizaki rats were higher than those in Wistar rats. Insulin levels in

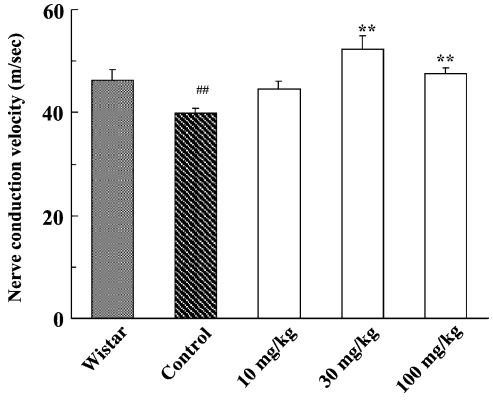

Fig. 5. Effect of JTT-608 on nerve conduction velocity in Goto–Kakizaki rats. JTT-608 was administered three times a day for 12 weeks. Caudal nerve conduction velocities were measured. Tail surface temperature was maintained at 37 °C with a heat pad. Data are means ± S.E.M. (Goto–Kakizaki rats; $n=7–8$, Wistar rats; $n=5$). $^{**}P<0.01$; significantly different from the control, Dunnett's two-tailed test. $^{\#\#}P<0.01$; significantly different from Wistar group, Student's *t*-test.

Table 1
Histopathological findings in kidney of Goto–Kakizaki rats that received chronic treatment with JTT-608

Findings	Grade	Goto–Kakizaki rat ($n=7–8$)				Wistar rat ($n=5$)
		Control	10 mg/kg	30 mg/kg	100 mg/kg	
		0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Increased mesangial matrix		0 6 1 0	1 5 1 0	2 6 0 0	1 6 1 0	5 0 0 0
Glomerular hyaline droplets		5 2 0 0	5 2 0 0	8 0 0 0	6 2 0 0	5 0 0 0
Thickening of glomerular basement membrane		3 4 0 0	5 2 0 0	5 3 0 0	5 3 0 0	5 0 0 0
Vacuolation of renal tubule		2 5 0 0	5 2 0 0	*7 1 0 0	*7 1 0 0	5 0 0 0
Hyaline cast		7 0 0 0	5 2 0 0	8 0 0 0	8 0 0 0	5 0 0 0
Tubular dilatation		6 1 0 0	4 3 0 0	6 2 0 0	7 1 0 0	5 0 0 0

Grade: 0, negative; 1, slight; 2, moderate; 3, severe. Incidence of each finding was analyzed with the non-parametric Dunnett's test.
* $P<0.05$; significantly different from the control.

the 100 mg/kg JTT-608 group showed a tendency to be lower than the control levels, but this did not reach significance (Fig. 1C). Triglyceride levels before administration of JTT-608 were about 130 mg/dl in controls, and the levels decreased to about 60–70 mg/dl after JTT-608 administration for 4 weeks. Triglyceride levels decreased dose dependently at 10 mg/kg JTT-608 or higher (Fig. 1D). Triglyceride levels decreased after 4 weeks of 10 mg/kg, 4–8 weeks of 30 mg/kg and during all treatment periods of 100 mg/kg JTT-608 administration.

The body weight of Goto–Kakizaki rats was lower than that of Wistar rats during the treatment period. Body weight in the JTT-608-treated group was not different from that of the controls (data not shown).

After 12-week administration, glucose levels at 30 min after meal loading and glucose areas above baseline from 0 to 2 h were reduced dose dependently by JTT-608 administration (Fig. 2). JTT-608 improved post-prandial glucose tolerance.

Glucagon-like peptide-1 levels in Goto–Kakizaki rats showed a tendency to a reduction compared to those in Wistar rats (mean ± S.E.M. values: 44.2 ± 2.1 vs. 52.7 ± 3.6 fmol/ml, respectively), but this did not reach significance ($P=0.08$). Glucagon-like peptide-1 levels in Goto–Kakizaki rats increased dose dependently after JTT-608 administration for 12 weeks and improved significantly at 100 mg/kg JTT-608 (Fig. 3).

3.2. Effect of JTT-608 on urinary protein

In the 5–12-week period, urinary protein levels in Goto–Kakizaki rats increased compared to those in Wistar rats. The levels in Goto–Kakizaki rats and Wistar rats were slightly variable for 5–12 weeks of administration. Urinary protein levels at each week decreased after administration of JTT-608 at 30 mg/kg or higher (Fig. 4).

3.3. Effect of JTT-608 on nerve conduction velocity

Motor nerve conduction velocities after administration of JTT-608 for 12 weeks in Goto–Kakizaki rats were markedly decreased compared to Wistar rats ones (mean ± S.E.M. values: 39.9 ± 1.0 vs. 46.3 ± 2.0 m/s, respectively). The levels for Goto–Kakizaki rats were improved on administration of JTT-608 at 30 mg/kg or higher (Fig. 5). The mean ± S.E.M. values at 10, 30 and 100 mg/kg for JTT-608

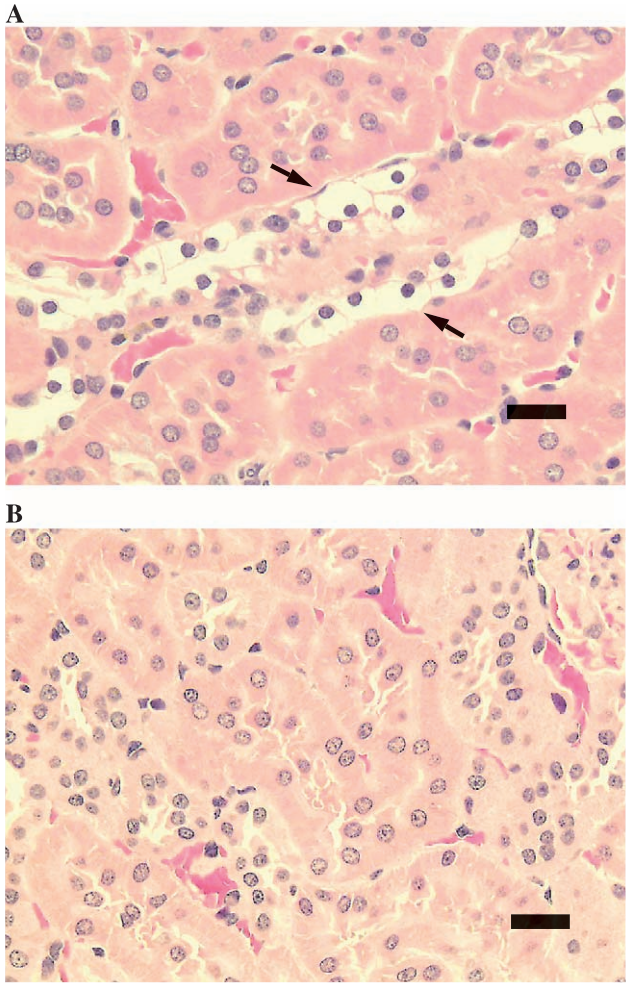

Fig. 6. Kidney (A) control, vacuolation of renal tubular epithelium (Armanni–Ebstein change, arrow), hematoxylin and eosin, bar = 20 μm. (B) JTT-608, 30 mg/kg. No abnormalities were observed in renal tubular epithelium, hematoxylin and eosin, bar = 20 μm.

Table 2
Histopathological findings in pancreas of Goto–Kakizaki rats that received chronic treatment with JTT-608

Findings	Grade	Goto–Kakizaki rat ($n=7–8$)				Wistar rat ($n=5$)
		Control	10 mg/kg	30 mg/kg	100 mg/kg	
		0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Atrophy of islets		0 6 1 0	0 7 0 0	0 8 0 0	1 6 1 0	5 0 0 0
Decrease in immunoreactivity of insulin		1 4 2 0	4 2 1 0	4 3 1 0	0 4 4 0	5 0 0 0
Focal fibrosis of islets		0 7 0 0	1 6 0 0	0 8 0 0	1 7 0 0	5 0 0 0
Hemosiderin deposit in islets		2 5 0 0	3 4 0 0	1 7 0 0	3 5 0 0	5 0 0 0
Focal cellular infiltration		6 1 0 0	7 0 0 0	8 0 0 0	7 1 0 0	5 0 0 0
Vacuolation of islet cell		6 1 0 0	6 1 0 0	7 1 0 0	8 0 0 0	5 0 0 0

Grade: 0, negative; 1, slight; 2, moderate; 3, severe.

groups were 44.6 ± 1.4, 52.4 ± 2.5 and 47.6 ± 1.1 m/s. Nerve conduction velocities in Goto–Kakizaki rats after administration of JTT-608 for 12 weeks recovered to the levels of Wistar rats.

3.4. Pathological analyses

Histopathological changes were observed in kidneys, pancreas, eyes and aorta of controls at all dose levels of JTT-608.

In the kidney of controls, changes due to diabetic nephropathy, such as increased mesangial matrix, glomerular hyaline droplets, thickening of glomerular basement membrane and vacuolation of renal tubule, were observed. Groups treated with 30 mg/kg JTT-608 or higher doses had a significantly reduced incidence of vacuolation of renal tubules (Table 1, Fig. 6).

In the pancreas of controls, histopathological changes such as atrophy of islets, decrease in immunoreactivity of insulin and focal fibrosis of islets, were observed (Table 2). Changes in the pancreas were also observed in the JTT-608 treatment groups, with no differences between groups. The pancreatic insulin content in Goto–Kakizaki rats decreased markedly compared to that in Wistar rats. The insulin content in JTT-608 treatment groups increased compared to that in the control group; however this difference did not reach significance (data not shown).

Sections of eyes and aorta in each group had no detectable differences.

4. Discussion

JTT-608, discovered as a result of screening at Japan Tobacco, stimulates insulin secretion in a glucose concentration-dependent manner and improves glucose tolerance (Shinkai et al., 1998). In the present study, we investigated the chronic effects of JTT-608 on fasting blood glucose level, hemoglobin A1c level and other diabetes related parameters in Goto–Kakizaki rats. JTT-608 was administered orally before feeding, three times a day, for 12 weeks. JTT-608 is rapidly absorbed and metabolized (Ohta et al., 1999a). In rats treated orally with JTT-608, the JTT-608 plasma concentration reached a peak at 0.25 h, after which it declined with a half-life of 0.43 h. Therefore we established an experimental system in which JTT-608 was administered 10 min before feeding of a liquid meal, in order to obtain sufficient effect from JTT-608. The Goto–Kakizaki rats were also treated with a liquid meal and JTT-608 simultaneously to approximate human meal and drug administration patterns. Blood glucose concentrations in diabetic rats were considered to be a good index for the chronic administration of JTT-608 (Fig. 1A and B). Administration of JTT-608 is thought to produce a decrease in hemoglobin A1c levels through suppression of the increase in blood glucose after daily meal-loading (Fig. 2). Hemoglobin A1c and triglyceride levels decreased in the control Goto–Kakizaki rats for 12 weeks (Fig. 1B and D). This appears to have been due to the change in meal pattern of the rats from ad libitum intake to a restricted intake (three times per day). Also, the composition of the liquid meal probably influenced triglyceride levels. JTT-608 showed an antidiabetic effect, as the drug ameliorated the abovementioned parameters. It has been reported that improvement of post-prandial glucose excursions in patients with type 2 diabetes is very important for blood glucose control (Kosaka et al., 1994), and that normalization of post-meal plasma glucose levels should be the target of rational therapy and a goal in early stages of the disease (Delprato and Tiengo, 2001). Furthermore, chronic blood glucose control suppresses the development of various complications in diabetic patients, as shown in the United Kingdom Prospective Diabetes Study. JTT-608 is thought to be useful, especially for inhibiting post-prandial blood glucose rise, and for providing good long-term glycemic control in treatment of patients with type 2 diabetes.

Since insulin resistance in Goto–Kakizaki rats has been reported (Sugiyama et al., 1989), the decrease of fasting blood glucose levels (Fig. 1A) may be attributed to improvement of insulin resistance by chronic glycemic control. Furthermore, the lipid parameter decrease (Fig. 1D) indicates that chronic glycemic control with JTT-608 improves not only glucose metabolism but also lipid metabolism. It has been reported that glucagon-like peptide-1

shows good effects on glucose metabolism, such as stimulation of insulin biosynthesis and release from pancreatic β cells, a deceleration of gastric emptying and an improvement in insulin sensitivity in peripheral tissues (Lam and Kieffer, 2002). An increase in glucagon-like peptide-1 levels with a high dose of JTT-608 (Fig. 3) is thought to be one mechanism by which chronic glycemic control is produced, but the mechanism underlying the increase in glucagon-like peptide-1 levels on JTT-608 administration is unknown.

To examine the effects of JTT-608 on diabetic complications, we measured urinary protein, and caudal nerve conduction velocity, in addition to pathological analyses of pancreas, kidneys, eyes and aorta. The urinary protein level in Goto–Kakizaki rats decreased after JTT-608 administration (Fig. 4). The histopathological changes in kidneys of Goto–Kakizaki rats were slight, as reported previously (Vesely et al., 1999; Yagihashi et al., 1978). JTT-608 treatment had an effect on pathology, showing an improvement of vacuolation of renal tubules (Table 1, Fig. 6). It is considered that chronic administration of JTT-608 arrests the development of renal lesions in Goto–Kakizaki rats. Histopathological findings other than the changes associated with diabetic nephropathy, such as hyaline cast and tubular dilatation, were observed but were considered accidental or spontaneous changes. Urinary microalbumin was not tested. It has been reported that Goto–Kakizaki rats show diabetic neuropathy from an early stage and caudal nerve conduction velocities in Goto–Kakizaki rats are lower than those in Wistar rats (Östenson et al., 1997; Goto et al., 1982). It is considered that the chronic glycemic control resulting from JTT-608 administration improves nerve conduction velocity or prevents the development of a decrease in the nerve conduction velocity. In Zucker diabetic fatty rats, motor nerve conduction velocity was improved by chronic glycemic control (Shibata et al., 2000). The authors measured nerve conduction velocity using an experimental method similar to ours. This method is thought to be useful for the evaluation of the effect on motor nerve conduction velocity. Nerve conduction velocity at a 30-mg/kg dose was higher than that with a 100-mg/kg dose (Fig. 5), because some animals in the 30-mg/kg group had high values. However, the difference in mean values between groups was not significant. Since pancreatic insulin content, relative volume and total mass of β cells in Goto–Kakizaki rats is low from an early stage compared to that of Wistar rats (Movassat et al., 1997), it was difficult to restore the insulin content and the organic lesions of the pancreas by JTT-608 administration for 12 weeks (Table 2). Histopathological findings other than the diabetic changes, such as hemosiderin deposit in islets, focal cellular infiltration and vacuolation of islet cells, were observed, but they are considered to be accidental or spontaneous changes.

In conclusion, JTT-608 is a novel antidiabetic agent that showed chronic glycemic control on administration before meals and gradually arrested the development of diabetic neuropathy and nephropathy.

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